Studies on the very low volume, controlled drop size application of MCPA, difenzoquat, paraquat and glyphosate.

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STUDIES ON THE VERY LOW VOLUME, CONTROLLED DROP SIZE APPLICATION OF MCPA, DIFENZOQUAT, PARAQUAT AND GLYPHOSATE.

Submitted by COLIN R. MERRITT for the degree of Ph.D of the University of Bath.

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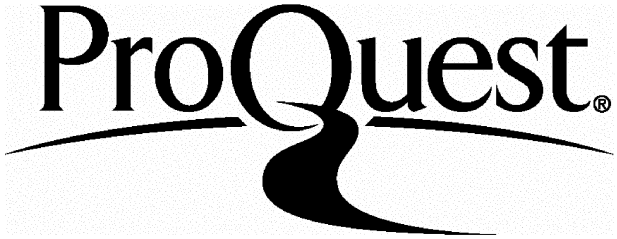

ABSTRACT

The effects of application factors on the performance of MCPA, difenzoquat, paraquat and glyphosate have been studied in relation to very low volume, controlled drop size sprays. Factors studied were drop size, herbicide concentration, position of deposit on the plant surface, and surfactant concentration.

Spray retention was measured on wild oat, radish and barley, comparing an application of around 20 $l.ha^{-1}$ using 250 um drops with a conventional spray of around 200 $l.ha^{-1}$. More herbicide was retained with the low volume rate application, but a greater proportion of that retained with the conventional spray was on the young and erect plant parts.

With MCPA and paraquat performance was unaffected by drop size between 200 and 400 µm, herbicide concentration or surfactant concentration. Glyphosate performance was not affected by drop size but was enhanced by higher concentrations of the herbicide. With difenzoquat, performance was impaired by higher concentrations and larger drop sizes due to necrosis at the site of treatment, which reduced entry and movement of the ^{14}C-labelled herbicide. Evidence is given that stomatal guard cells are preferential sites of entry of exogenously applied chemicals, and it is suggested that this may contribute to the onset of necrosis by damage to the stomatal mechanism. Difenzoquat performance was also reduced by a low surfactant concentration.

The effects of position of deposit were specific to each herbicide. On radish, paraquat was most effective when applied to the cotyledons whilst MCPA was most effective on the midvein of the foliar leaves, and glyphosate when applied to the foliar leaf laminae between veins. Paraquat and difenzoquat were most effective on younger leaves of wild oat and towards the lamina base, whilst leaf age did not influence the performance of glyphosate with this species.

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CONTENTS

1. INTRODUCTION

The methods of herbicide application used in agricultural practice have changed little over many years (See Section 2.1). Conventional spraying is based on hydraulic nozzles which operate at 0.7 - 3.5 bars pressure to apply 100-500 $l.ha^{-1}$ of spray liquid.

In recent years, particularly since 1974, considerable interest has been shown in methods of herbicide application using much lower volumes of spray liquid per unit ground area, whilst maintaining the same dose of herbicide per unit area. These methods were made possible by the use of techniques which produce sprays of relatively controlled drop size, and such methods have become known by the collective term controlled drop application, or CDA (British Crop Protection Council, 1978). Controlled drop application has been the subject of extensive evaluation in field experimentation by government and industrial research groups in the U.K., leading to the general conclusion that herbicides which are well translocated within plant tissues are about as effective at volume rates of around 20 $l.ha^{-1}$ as at more conventional volume rates. Herbicides which are poorly translocated (the so-called 'Contact' herbicides) are generally less efficacious as volume rates are reduced. The distinction is less clear cut with some herbicides, whose suitability for CDA varies according to such factors as season and climate.

Although retention appears to favour reduction in volume rates (See Section 2.3.2) few instances of improved herbicide performance by controlled drop application at very low volume rates have been observed. To date the reasons for differences in efficacy between CDA and conventional herbicide applications have not been adequately identified.

Changes in method of herbicide application might be expected to affect several of the processes in the sequence from emission of spray

from the atomiser to the final effects of the herbicide molecule at its site of action in the plants. These changes would probably influence the magnitude of losses of effective herbicide occurring during the application sequence as indicated in Table 1.1, so that study of such quantitative effects should identify the causes of differences between application methods.

The present study was intended to provide information on the factors responsible for differences in herbicide performance due to application variables. It was also intended that the work should be relevant to controlled drop application at very low volume rates, with some comparison between this type of application and conventional spraying. The variables considered to be of most importance for this study were drop size, herbicide concentration, surfactant concentration and the position of the herbicide deposit on the plant surface. Of these variables the first three are under the direct control of the operator of spray machinery with CDA equipment. The variation in concentration of active ingredient and of additives between the two types of spraying is especially marked and might be expected to produce differences in effectiveness. With these first three variables the effect on biological efficacy of the herbicides was considered to be the important starting point for investigation, and from this point of view techniques were necessary to isolate these effects from others such as retention, thus confining the factors involved to processes C and D in Table 1.1.

With the fourth variable, position of the herbicide deposit, it was necessary to consider two aspects; firstly the effects of changing the application on the positions of the retained spray deposits, and secondly the differences in biological performance of herbicide

Table 1.1 Processes in the Sequence from emission of spray from the atomiser to the final effects of the herbicide at its site of action, and lossess of active ingredient during this sequence.

	Process		Losses
A	Movement of drops from spray machine to target zone.	1.	Drift of spray out of target area.
		2.	Interception by crop or other non-target obstacle.
B	Impaction and retention of spray on target surface.	1.	Spray drops missing target.
		2.	Spray drops reflected from target surface.
		3.	Spray liquid leaving target surface by "run-off" following coallescence of drops.
C	Entry of active ingredient into plant tissue.	1.	Left on plant surface, either in carrier or after evaporation of carrier.
		2.	In an unavailable state in non-cellular surface layers.
		3.	Washed off by rain or other precipitation before entry can take place.
		4.	Blown or shaken off by wind before entry can take place.
		5.	Breakdown of a.i. on surface (eg by oxidation, photodecomposition).
		6.	Redistribution of a.i. on surface.
		7.	Volatilization of a.i.
D	Movement of active ingredient to site of action.	1.	Remaining in tissue adjacent to site of entry.
		2.	Moving to another site (eg by apoplastic movement in transpiration stream).
		3.	Detoxication by plant.
		4.	Removal from transport system *en route*.
		5.	Movement blocked *en route*.

deposits at these various positions. The varying results of field and laboratory experiments with herbicides have often been ascribed to the positional aspects of the application, but there is little supporting evidence. For example reduced effectiveness is often attributed to poor 'cover', a term used arbitrarily to describe the degree of dispersion of spray over the target surface. Also reference is often made to treatment of the 'growing point', assuming this to be beneficial. The relatively small literature on this topic is reviewed in Section 2.2.

Four rather different foliage-applied herbicides were chosen for this investigation (See Section 3.2). MCPA and difenzoquat are used selectively, mainly in cereals, for the control of dicotyledonous weeds and wild oats respectively, and both have been the subject of recent research on CDA (See Section 2.1). Paraquat and glyphosate were the other herbicides chosen, because both can be used for total weed control and could therefore be used on the same species as MCPA and difenzoquat. This allowed comparisons between herbicides to be made without complication due to differing plant material. Paraquat, which is usually poorly translocated, differs markedly from the well-translocated glyphosate. This difference was expected to be of importance in determining suitability of the herbicides to low volume application.

Having identified the influence of application variables on the biological efficacy of these four herbicides it was the intention of this study to go some way towards explaining the causes of such effects. The entry and movement of herbicides were thought to be likely areas of profitable study and some work has been carried out on this topic. No attempt was made, however, to study biochemical aspects of herbicide mode of action since it was felt unlikely that application variables would affect such processes other than through changes in the amounts of herbicide entering and moving within the plant.

2. LITERATURE REVIEW

2.1 Controlled drop and low volume application

Rose (1963) described numerous machines using relatively low volume rates, including high speed air jets and rotary devices used by the pioneers of modern chemical crop protection to apply oils and pyrethrins for the control of insect pests by "concentrate spraying" methods during the 1920's and 1930's. Subsequently concentrate spraying, or ultra low volume (ULV) spraying has generally been associated with insecticide application, where typically very small drop sizes are drifted or blown with air currents onto the target (Maas, 1971). Maas also describes the special formulation requirements of ULV spraying, such as the use of low-volatility carrier liquids to ensure drop longevity in evaporative conditions when using these very small drops.

This "traditional" form of ULV spraying has not been widely adopted for herbicides, largely because of the danger of herbicides drifting onto sensitive crops or natural vegetation outside the sprayed area. However, in extensive situations where these dangers are reduced ULV herbicide spraying has been successful, notably in forestry where woody species and heather (Calluna vulgaris) have been controlled with a 2,4,5-T and 2,4-D mixture, and bracken with asulam, using hand-held spinning disc sprayers (Brown and Thomson, 1974; Rogers, 1975).

In arable uses of herbicides, spray volumes have ranged from less than 150 $l.ha^{-1}$ to greater than 500 $l.ha^{-1}$, but are generally between 200-300 $l.ha^{-1}$ (Cussans and Taylor, 1978; M.A.F.F., 1976). Recent surveys have shown that such high volume rates require much time and energy for water haulage and tank refilling (M.A.F.F., 1976; Byass and Lawrence, 1976) suggesting that a reduction in volume rates could have economic advantages over present methods.

A reduction in spray volume rates would help in a number of ways. Firstly it would ensure better use of the limited time period during which herbicides can be safely applied to cereal crops (Evans, 1974), especially in winter cereals where the poorer weather conditions further restrict the time available for spraying. A reduction in volume rate could render more days suitable for spraying when problems of access to wet land would restrict normal spraying; the combination of low volume rates with special lightweight spray vehicles may be of importance here (Cussans and Ayres, 1978). Other weather conditions, such as wind and rain, may also restrict the time available for spraying (Adams, 1978; Tottman and Phillipson, 1974) and here the timeliness of low volume applications would be of value. The potential for reduced weight of the total spray vehicle could result in reduced soil compaction and less damage to the growing crop by wheel marks. Finally expensive spray additives which may benefit herbicide performance could become economic when volume rates can be substantially reduced. (Taylor and Holly, 1976).

It is generally accepted that some control over drop size is necessary to maintain uniformity of distribution of spray at very low volume rates, and to avoid wasted spray in the form of either drops which are too small and drift from the target area or those which are too large to be retained on weed foliage. Hence much of the previous work on very low volume applications of herbicides has been with sprays of uniform or narrow spectrum drop sizes. Rose (1963) describes early studies by E. J. Bals on the use of rotary atomisers to produce such uniform drop sizes for pesticides, and rotary atomisers are also widely used in the chemical engineering industry for spray drying, as reviewed by Dombrowski and Munday (1968).

The most commonly employed rotary atomisers in herbicide research have been spinning discs, although a device comprising four rotating tubes was developed by Amchem Products in the U.S. which produced a relatively large drop size of 540 μm mean diameter, which was designed primarily for soil-applied herbicides.

In addition to rotary atomisers, nozzles have also been designed that produce relatively uniform drop sizes, and some success has been achieved with so-called "magnetostrictive" nozzles in which piezoelectric crystals create cyclic disturbances causing a regular breakup of liquid streams (Bouse, Haile and Kunze, 1974).

Only in recent years has rigorous biological experimentation with low volume spraying been attempted using controlleddrop size sprayers. Sokolov et al, (1970) described work using spinning discs to apply oil-based solutions of 2,4-D ester with promising results. Later work confirmed these results using 2,4-D ester in deisel fuel oil as the carrier liquid, and also showed that water-based formulations of 2,4-D amine for barley crops and MCPA sodium for flax could be successfully applied at 6-25 and 25-50 $l.ha^{-1}$ respectively (Sokolov et al, 1974). Barzee and Stroube (1972), using the Amchem rotary atomiser, applied a range of pre-emergence herbicides at volume rates of 9.35 and 37.4 $l.ha^{-1}$ and concluded that such herbicides could be as effective at these very low volume rates as at the conventional rate of 187 $l.ha^{-1}$. Experiments with magnetostrictive nozzles reported by Buering, Roth and Santleman (1973) showed that foliage-applied herbicides, including paraquat, gave better control of weeds at higher volume rates and with smaller drop sizes.

In Britain preliminary work to assess the potential of very low volume application of herbicides at the Weed Research Organization has

been reviewed by Cussans and Taylor (1976). In the original studies commercially available spinning discs intended for the application of insecticides at ULV rates were used, but at lower rotational speeds to produce relatively large drops of 280 μm. To overcome problems with the uneven distribution of drops across the swath the discs were shrouded, and spray contributing to the outer portion of the swath captured and recycled. The discs employed could only produce uniform drops with oil solutions, so the oil-soluble herbicides barban, 2,4-D ester and triallate were applied in oil carrier liquids in comparison with conventional applications, which were emulsions in water. The results suggested that these herbicides could be applied at very low volume rates in this manner without major loss in effectiveness (Taylor and Merritt, 1974). Later the production of a spinning disc which could atomise water-based sprays uniformly at practical flow rates led to improved designs (Taylor, Merritt and Drinkwater, 1976) involving stacking of shrouded discs to provide an even spray distribution across the swath and higher output without the need to recycle spray. This design in turn led to the development of a commercial trials sprayer (Hind, 1978) and a commercial tractor-mounted farm sprayer (Farmery and Peck, 1976). Glasshouse experiments at the W.R.O. were conducted to assess the suitability of a wide range of foliage-applied herbicides for application at volume rates between 5 and 45 $l.ha^{-1}$ using drop sizes between 150 and 350 μm, a range that has become known as controlled drop application or CDA (Merritt and Taylor, 1977). These glasshouse experiments showed that some herbicides were as effective at very low volume rates as at conventional rates, whilst others were markedly reduced in performance as volume rate was lowered. In general those herbicides which were less effective within the CDA range were contact herbicides, such as bentazone and the mixture of ioxynil and bromoxynil, which are

only translocated in plant tissues to a limited extent and therefore mainly affect only those plant parts which retain the spray. Herbicides which were known to be well translocated generally performed well at very low volume rates, although some, such as the phenoxypropionics, mecoprop and dichlorprop, were in an intermediate category with some reduction in performance by CDA.

A programme of field experiments followed the initial glasshouse study. A dicamba mixture performed as well with CDA as with conventional methods for dicotyledonous weed control in cereals, whilst an ioxynil mixture was less effective, agreeing with the earlier findings (Ayres, 1976; Ayres and Merritt, 1978). Barban was as effective for wild oat control at 20l. ha^{-1} as by conventional spraying at 175 l. ha^{-1}, whilst difenzoquat produced somewhat variable results (Wilson, 1976; Wilson and Taylor, 1978; Ayres, 1978a). In the case of difenzoquat it appeared that differences between seasons and dates of application occurred with CDA treatments. The differences may have been due to weather conditions or to crop canopy interception of spray (Ayres, 1978a). Work with glyphosate on Agropyron repens (Couch) showed that this herbicide was consistently more effective with CDA. (Turner and Loader, 1978; Caseley, Coupland and Simmons, 1976). Finally work with soil-applied herbicides for dicotyledonous weed control on organic soils (May and Ayres, 1978) and blackgrass control (Ayres, 1978b) gave results which were similar for CDA and conventional applications, although there was a suggestion of poorer results with CDA at the later post-emergent date, possibly due to an increase in the importance of foliar activity.

Following the introduction of commercial trials and farm CDA machines many more comparisons between CDA and conventional spraying

were made, and some reports suggested equivalent weed control by CDA and conventional methods (Grosjean and Cook, 1978; Mayes and Blanchard, 1978) whilst others showed that CDA produced poorer results (Bailey and Smart, 1976; Bailey et al, 1978; Harris et al, 1978; Robinson, 1978). However the results largely depended on the herbicides used since many of the poorer results were obtained with the contact or semi-contact herbicides. The general consensus from all this field evaluation remains that most herbicides, excepting the purely contact ones, perform about as well, or slightly less effectively with CDA as with conventional spraying, and that the agronomic benefits from reduced volume rates make CDA an attractive technique. However, there remains a lack of published research on the more fundamental aspects which might help to explain the observed field and greenhouse results in terms of the effects of changing application method on herbicide performance.

2.2 The importance of form of deposit

It is well established that the physical properties of sprays affect the amount of herbicide retained by plant surfaces, and thereby influence herbicide performance. There is much less published information on the importance of the form of the herbicide deposit in determining the biological effect. This lack of study is partly due to the practical difficulties of such work, particularly of producing uniform drops of sizes normally encountered in agricultural sprays and applying them to plant surfaces in a predetermined manner.

Studies of drop size, and of drop density (volume rate) have been achieved by spraying whole plants, usually with equipment based on spinning discs to provide controlled drop sizes, but often the influence of retention has not been determined or isolated from effects due to the form of deposit. Ennis and Williamson (1963) used an elaborate shuttered spinning disc device which was capable of applying a chosen dose to a chosen area in uniform drops. They used this to study the effects of drop size on the reduction in yield of a range of crop species by several herbicides, including 2,4-D. With all herbicides small drops (about 100 μm) were more effective than large drops (about 300 μm). They suggested that the differences were due partly to the larger drops becoming physiologically isolated due to profound effects on cells immediately beneath the drops, and partly to the greater likelihood of small drops achieving direct contact of chemical with sites of greatest activity, such as the stem growing points. McKinlay, Brandt, Morse and Ashford (1972) reported similar results with 2,4-D on sunflower comparing drops of 100-400 μm, applied with a spinning disc. Work with paraquat on sunflower (McKinlay, Ashford and Ford, 1974) again suggested that smaller drops (100 μm) were more effective

than larger drops (350 μm) but also that lower volume rates (ie higher concentrations) were more effective. Douglas (1968) applied well-spaced drops from a spinning disc to bean leaves and assessed the influence of drop size on diquat and paraquat toxicity by measuring lesion diameter. By this method he found that an optimum drop size occurred for both herbicides, this being about 400 um for paraquat and about 500 μm for diquat. However when he sprayed whole Polygonum aviculare plants with paraquat he found that toxicity increased with decreasing drop size down to 250 μm. He ascribed this effect to retention because the contact angle of drops on P. aviculare leaves was high. Buering, Roth and Santleman (1973) used magnetostrictive nozzles to study the effects of drop size on paraquat, MSMA and fluometuron activity on dicotyledonous weeds, and found in each case that smaller drops and higher volumes were more effective, although their smallest drop size was 400 μm and these were compared with very large drops up to 860 μm. Retention was not considered as a factor in these experiments, but it may well have had a major influence. Lake and Taylor (1974) compared drop sizes of 100-400 μm and volume rates up to 100 $l.ha^{-1}$ of barban on wild oat. They measured retention and found this to be linearly related to volume rate with no effect of drop size. Their results showed that a given volume of the smaller drops was more effective in reducing the growth of wild oat than an equal volume of the larger drops.

The effect of position of the herbicide deposit on plant surfaces has generally been investigated using microsyringes to apply the drops, but the smallest drops which can be produced and placed on leaf surfaces are about 0.2 μl (726 μm) and in practice much work with microsyringes has involved even larger drops. Mullison (1953) applied

a range of concentrations of 2,4-D to bean plants in drops of 2-6 ul. Neither the drop sizes or concentrations used affected 2,4-D activity at a given dose. Holly (1954) compared a range of different positions of treatment of linseed and sunflower using MCPA and 2,4,5-T applied in drops of 5 µl. Herbicide performance was not affected by most positional variations tested, including proximal and distal ends of cotyledons and cotyledon axils. Treatment to the second leaf pair of sunflower was slightly more effective than treatment to the first leaf pair, whilst treatment of the unopened terminal bud was less effective. It was suggested that the treatment to the terminal bud involved a smaller area of contact, which may partially explain its poorer effectiveness as a site of application, whilst differences between the first and second leaf pair were best explained in terms of translocation since there were similar degrees of necrosis caused by treatment to these two positions but the second leaf pair was closer to the actively growing terminal node.

There are several reports of work with wild oat herbicides in relation to position of deposit. Holly (1960) found that barban was more effective when placed at the leaf base and leaf sheath than at the leaf tip. Similar results were obtained by Neidermeyer and Nalewaja (1974) and Coupland, Taylor and Caseley(1978) using wild oats at different stages of growth. Other wild oat herbicides were found to be more effective at leaf bases and on leaf sheaths, including chlorfenprop-methyl, benzoylprop-ethyl, HOE 23408 defenzoquat and flamprop-methyl (Hack, 1973; Bischof and Walter, 1974; Walter and Bischof, 1976; Coupland, Taylor and Caseley, 1978). Walter and Bischof (1976) suggested that the amount of epicuticular wax at the site of treatment may be largely responsible for observed differences in herbicide performance, a view shared by Coupland et al, (1978). The latter also found differ-

ences between applications to leaves of different ages. Generally the youngest fully expanded leaf was the most effective site, with younger and older leaves being less effective. These differences were attributed to the ability of the leaves to export assimilates and herbicides. The work of Coupland et al, (1978) also included a study of position of glyphosate treatment on three-leaf couch plants (Agropyron repens). Here there were no differences between leaf age and it was suggested that this may be due to glyphosate being more readily translocated, or possibly to differences in the translocation systems of wild oat and couch.

2.3 Plant surfaces in relation to herbicide performance

There have been many studies of plant surfaces including their chemical and physical nature and effects on the retention and spreading of sprays as well as the penetration of substances into plant tissues. Several recent reviews, which emphasize different aspects, have been consulted including those by Currier and Dybing, 1959; Linskens, Heinen and Stoffers, 1965; Franke, 1967; Hull, 1970; Kirkwood, 1972; Bayer and Lumb, 1973; Hull, Morton and Wharrie, 1975 and Sargent, 1976, but that by Martin and Juniper (1970) has been most valuable.

2.3.1 Chemistry, structure and properties of plant surfaces

The surfaces of the aerial parts of plants possess many features visible to the naked eye, such as trichomes and veins, plus others only detectable by light and electron microscopy. All of these features can influence the results of herbicide application, as can the cuticle itself, which covers the entire plant surface, including the sub-stomatal cavities; it comprises several more or less distinct layers.

On the outer surface of the cuticle, and to some extent within the cuticle, waxes are often present, which show a range of morphological forms and crystalline structures, although this epi-cuticular wax may be entirely absent in some species (Schieferstein and Loomis, 1956 and 1959; Eglington and Hamilton, 1967). What determines the form of this wax is not yet fully understood. It was thought that wax morphology was determined by the way the waxes were extruded (Hall, 1967; von Wettstein-Knowles, 1974), indeed structures resembling pores and channels in the cuticle have been observed through which wax could be extruded as precursors or in some volatile carrier (Hall, 1967; Fisher and Bayer, 1972). However, evidence now suggests that the

chemical composition may be the major factor determining the morphology of the wax (Jeffree, Baker and Holloway, 1975).

Cuticular waxes vary greatly between species in their chemical composition, and include hydrocarbons (mostly alkanes), alcohols (including diols), Ketones (including diones), fatty acids, hydroxy-fatty acids and esters (Martin and Juniper, 1970). Some waxes contain aldehydes and cyclic compounds such as terpenoids. Chain lengths are typically 21-35 carbon atoms for hydrocarbons, alcohols and Ketones and 16-18 atoms for fatty acids. The function of the epicuticular waxes and the outer surface of the cuticle is largely protection, against water loss, ultra-violet radiation, high temperature and abrasion by wind-blown soil particles or damage by heavy rain. The chemistry and morphology of the surface waxes largely determine the resistance of the plant surface to water loss and thus influence the wetting characteristics. The wetting and spreading of liquids on plant surfaces are often characterised by the angles of contact at the liquid/solid/air interface. Fogg (1948) showed that contact angles of water on leaf surfaces varied diurnally due to the water content of the tissue. He suggested the variation was due to changes in the corrugation of the surface, since surface topography or roughness is known to influence the contact angle (Wenzel, 1946). Holloway (1969, 1970) found no definite correlation between the contact angles of leaf surfaces and of their individual chemical constituents; the contact angles of water on the waxes of 40 species were between 92 and 107° whilst on the intact plant surfaces they ranged between 40 and 170°. Hence the crystalline structure seems to be more important than the chemical composition, presumably by introducing air films below the liquid drop, making the surface a composite of air/liquid and solid/liquid interfaces. The effect of contact angle on spreading was studied theoretically by Ford and Furmidge (1968) who

pointed out that impact energy, governed by drop size and velocity, will also affect the extent of drop spread.

A layer composed largely of cutin, a polymerised form of hydroxy-fatty acids in a three-dimensional polyester network, lies immediately beneath the epicuticular waxes. This layer has been termed the 'cuticle proper' (Martin and Juniper, 1970) and in addition to cutin contains some embedded waxes and other constituents such as tannins and suberin. The properties of this layer are determined by the largely lipoidal nature of cutin so that lipid-soluble substances may be expected to traverse the layer most readily, although Crowdy (1959) has pointed out that polar properties due to the presence of Carboxylic acid groups in the cutin may cause it to absorb water and swell, thereby providing a relatively hydrophilic route.

Between the cuticle proper and the cell wall there exists in many plants a layer containing pectic substances, and thought to be continuous with the middle lamella of the anticlinal walls of the epidermal cells (Martin and Juniper, 1970). Pectic substances contain a mixture of polysaccharides of which the largest component is the linear molecule of $\alpha 1 \rightarrow 4$ linked D-galacturonic acid residues, in which some of the carboxyl groups are methylated. In many species there also appears to be a distinct layer between the pectin layer and the cuticle proper, which may be termed the 'cutinised layer' and contains in addition to cutin some cellulose fibrils extending from the cell wall (O'Brien, 1967; Hallam, 1967).

The cell wall lies between the pectinaceous layer and the plasmalemma (the outer membrane of the cytoplasm) and is composed of a matrix of cellulose fibrils embedded in a matrix of hemicelluloses and pectic substances (Goodwin and Mercer, 1972). Cellulose is a

polysaccharide consisting of $\beta 1 \rightarrow 4$ linked glucopyranose residues, and is readily hydrated so that the cell wall forms a substantial part of the aqueous, extra-cellular volume of plant tissues (the apoplast), the site of the transpiration stream. Thus in crossing from the outer surfaces of leaves to the living cells, substances must pass through the largely hydrophobic cuticular layers and the hydrophilic pectinaceous layer and cell wall (Bayer and Lumb, 1973). The final boundary which must then be crossed is the plasmalemma, which is composed of phospholipid molecules, probably largely in a bimolecular layer, with associated protein molecules, both on the surface and embedded within the lipid (Conn and Stumpf, 1972). A number of possible mechanisms have been proposed for transport across the plasmalemma; it is known that in addition to simple diffusion, active processes are involved, and that the plasmalemma, like other membranes, exhibits selectivity in its permeability to substances.

2.3.2 Impaction, retention and spreading of spray drops on leaf surfaces

The efficiency of a given application depends in part on the amount and distribution of spray impacting on and being retained by the plant surfaces and also on the subsequent behaviour of the retained spray drops.

Retention has been measured under a variety of conditions using several methods. Fogg (1947) determined retention by weighing plants before and after spraying. Many workers have added coloured dyes to the spray liquid and determined retention spectrophotometrically after washing the plants (Holly, 1954; Woofter and Lamb, 1954; Brunskill, 1956; Blackman et al, 1958; Furmidge, 1962a; Hibbit, 1969; Lutman and Sagar, 1975; Sharma et al, 1978). Others have used fluorescent

dyes in a similar way (Sharp 1974, 1976; Lake and Taylor, 1974; Lake, 1977; Merritt and Taylor, 1978). Davies et al, (1967) measured ioxynil retention directly by ultra-violet spectrophotometry of the ioxynil that remained on the leaf.

Most of the work reported above involved the measurement of retention of sprays from conventional hydraulic nozzles. The contribution to selectivity of differential retention of herbicide between relatively waxy crop species such as cereals, flax and peas, and less waxy weed species, was studied by Holly (1954), Blackman et al, (1958), Davies et al, (1967), Hibbit (1969) and Sharma et al,(1978). In general this work showed that retention differences could account for a large proportion of the observed selectivity, and that lowering the surface tension by addition of wetting agents reduced selectivity by increasing retention on waxy species but not on less waxy species. Blackman et al, (1958) also showed that a reduced mean drop size could increase retention, again causing reduced selectivity. Blackman et al, (1958) and Woofter and Lamb (1954) showed that the growth stage of plants affected levels of retention. Furmidge (1962a) studied retention on artificial targets of beeswax and cellulose acetate at the point of maximum retention, known as the point of "incipient run-off", and derived a retention factor based on the dynamic advancing and receding contact angles of the spray liquid on the target surface, and the liquid surface tension. This factor could be used to predict the maximum retention value. In a second paper (Furmidge, 1962b) leaf surfaces were used; whilst retention was proportional to the retention factor on a given species, considerable variation occurred between species. Furmidge believed that the differences were mainly due to variations in surface topography, or "roughness" at macroscopic and microscopic levels.

Brunskill (1956) studied the retention of individual drops on pea leaves. Retention was increased by reduction in drop diameter, reduction in surface tension and increase in drop velocity, whilst viscoity seemed relatively unimportant. The importance of surface tension was demonstrated by using a range of concentrations of methanol and acetic acid. It was shown that at the surface tension of pure water (about 72 mNm^{-1}) retention decreased at drop sizes greater than about 100 μm, whilst with a surface tension of about 40 mNm^{-1} drops of 250 and 350 μm were completely retained. The use of surfactants to reduce surface tension has to be treated with some caution, however, since as Hartley (1966) pointed out the dynamic surface tension, such as at a newly formed surface during the impaction of a drop, may differ from the measured static surface tension, due to the time taken for large surfactant molecules to diffuse towards and concentrate at, the liquid-air interface. In a study of the impaction process Ford and Furmidge (1968) revealed that the times involved in the process of impaction were sufficiently short for dynamic surface tension effects to influence the result. Such effects may well explain the observed increases in retention which occur beyond the critical micelle concentration of surfactant solutions, when the surface tension is at a minimum (Davies et al, 1967; Hibbit, 1969).

Sprays produced by spinning discs, with drops of uniform sizes falling vertically at or below terminal velocity have been used to study the effects of drop size and volume rate on retention. Using an oil-in-water emulsion Lake and Taylor (1974) found that retention on wild oat was directly proportional to volume rate up to 150 $l.ha^{-1}$, with no difference between drop sizes in the range 110-440 μm. However, with an aqueous solution of 'Agral' surfactant (0.1% v/v) Lake (1977) found that retention by young wild oat and barley plants was lowered

at drop sizes above 110 μm. Merritt and Taylor (1978), working with a higher concentration of Agral (0.5% v/v) found that wild oats retained 150 and 250 μm drops equally well, but there was poorer retention of 350 μm drops.

In addition to characteristics of the leaf surface, formulation and spray type, plant morphology, in particular leaf angle, has been found to be of importance. Brunskill (1956), Davies et al,(1967), Hibbit (1969) and Lake (1977) all found that retention increased as leaf angle approached the horizontal, although the results are largely explained by the projected plan area which also increases as the horizontal is approached. This is particularly well demonstrated by the results of Lake (1977) who expressed retention of a plan area basis. Lutman and Sagar (1975) proposed that leaf angle was a major factor causing an increase of retention by oat plants grown at high nutrient status.

Whilst the total retention by plants is undoubtedly important, the distribution of retained spray on the plant surface may be of equal or greater importance when there exist differences in the susceptibility of a plant to a herbicide according to the site of deposition. Using sunflower, Blackman et al, (1958) showed that although the bulk of retained spray remained on the main leaves, a lowering of surface tension markedly increased the proportion on the shoot apex, which might be expected to affect herbicide performance. Hibbit (1969) found that when wild oats were sprayed with a solution devoid of surfactant most of the retained spray was located at the leaf base, and flax retained most spray on the cotyledons; both of these sites had previously been shown to be more effective sites of application of asulam (Hibbit, 1967). It therefore seems likely that the

distribution of retained spray could be a major factor in herbicide performance.

The form of a spray deposit on a plant surface will be affected by the degree to which the drops spread. Ford and Furmidge (1966) who discussed drop spread in terms of the energy considerations of the impaction process and of the physical properties of the spray liquid, derived a formula for the spread factor (the ratio of the radius of the wetted area to that of the original drop), which depended on the contact angle at the air/liquid/solid boundary. Johnstone (1973) used the spread factor to determine the fractional cover (defined as the wetted area per unit area of surface) for a range of drop sizes and contact angles, showing that the fractional cover is inversely proportional to these properties. Thus reduction in surface tension and drop size will lead to greater area of contact of a given amount of spray.

Verification of these calculations using actual spray drops on leaf surfaces has been limited by techniques available, but such practical study has recently become possible using the scanning electron microscope in conjunction with techniques of Cathodoluminescence (Hess, Bayer and Falk, 1974), X-ray fluorescence (Falk, Hess and Bayer, 1975); or by direct viewing of the deposit by Seaman (1979) who found that, using a surfactant solution, the initial drop retracts as evaporation of water proceeds until the final form of the deposit consists of smaller drops and smears of the drop contents after most of the water has evaporated, apparently confined to preferentially wetted areas.

2.3.3 The penetration of substances into plant tissues

The influence of leaf surface factors on the penetration of pesticides and plant nutrients has been studied using both isolated

cuticles and intact leaves. Cuticles may be isolated mechanically, by abrasion of underlying tissue or peeling, or chemically by boiling with ammonium oxalate and oxalic acid, or by the technique which is now generally preferred, using enzyme preparations to dissolve the pectic layer (Norris and Bukovac, 1968). Goodman and Addy (1963) using chemically isolated cuticles, concluded that size, ionic charge and spacial configuration of different compounds had no influence on their penetrability. Work by Norris and Bukovac (1968, 1969, 1972) suggests that penetration through isolated cuticles of 1-Naphthaleneacetic acid operates largely by diffusion at fairly low penetration rates, although they suggest that this may be a feature of constant volume aqueous penetration methods, because when droplets were allowed to evaporate, penetration increased as concentration increased. The high Q10 value observed for cuticular penetration was attributed to the lipoidal nature of the cuticle and changes in its physical character that occurred at the higher temperatures. Removal of surface wax also caused a marked increase in penetration, and finally they concluded that the observed rates of penetration by these methods could account for physiologically significant amounts of chemical entering plants, thus obviating the need for other specific routes of entry. The range of permeabilities shown by isolated cuticles is very wide, as shown by the work of Darlington and Cirulis (1963) who cited penetration rates for apple cuticle between about 2% for sucrose and glucose and about 60% for certain α-chloroacetamide derivatives. More recent work with isolated orange leaf cuticles showed that permeability coefficients of two herbicides, an insecticide and a fungicide were between 0.32 and 1.5 that of tritiated water (Davis, Mullins, Stolzenberg and Booth, 1979), although these authors remarked on large variations in their data; it seems that a major problem with the use of such isolated

cuticles is the acquisition of reproducible samples of cuticle.

Several authors have acknowledged the limitations of isolated cuticle studies; both the rate and amount of penetration are likely to differ in intact leaves due to the presence of other barriers, particularly the plasmalemma, which may decrease the concentration gradient across the cuticle by limiting the movement of absorbed substances away from the point of entry. Conversely any binding of substances to internal components or active removal from beneath the cuticle could increase the concentration gradient and thereby increase penetration rates. Further doubts about isolated cuticle studies arise due to possible effects of the isolation method on cuticle permeability and to the somewhat artificial system of constant volume receiver and donor solutions normally favoured.

Sargent and Blackman (1970, 1972) and Sargent (1976) describe studies on the penetration of 2,4-D and chloride ions into leaf discs or detached whole leaves of Phaseolus vulgaris and other species, which suggest some active component of penetration. They found that penetration was increased by light, has a high Q10 and is decreased in light by the addition of metabolic inhibitors. The effect of light was shown not to be directly due to the opening of stomatal apertures, although the presence of stomata did appear to increase penetration; it was concluded that guard cells and subsidiary cells might be preferential sites of entry. Blackman and Sargent used non-drying constant volume donor solutions, usually in reservoirs attached to leaves or as agar blocks, which makes their results difficult to compare with those from application of drops as in normal spraying.

Fang (1958) studied the entry of 2,4-D by applying drops to leaves on intact plants and found that half or more of the applied

dose did not enter the leaf and 85-90% of that which entered then remained in the treated leaf. It has often been observed that when herbicides are applied in this way most of that which enters does not then move much further, as judged by surface washings and assaying both the washings and the treated area or leaf. This has been shown for MCPA and MCPB (Kirkwood et al, 1972), paraquat (Bland and Brian, 1975) and difenzoquat (Sharma, Vanden Born, Friesen and McBeath, 1976). Glyphosate does not follow this pattern and significant amounts are translocated to regions of high metabolic activity, although substantial amounts still remain in the treated leaf (Sprankle, Meggitt and Penner, 1975). The reason for this concentration of absorbed chemical at the treated site is not yet known. The possibility that poor penetration of the plasmalemma is the cause seems unlikely because it has been shown that several pesticides which appear to move apoplastically do enter the living cells but are not transported in the symplast simply because they diffuse out again as readily as they enter (Peterson and Edgington, 1976).

Many attempts have also been made to find specific pathways of entry, and the possible role of stomata in particular has been examined. It has been established that differences in penetration rates between leaf surfaces, both within and between species, are correlated with the presence and density of stomata (Turrel, 1947; Sargent, 1965; Jung, Wittwer and Bukovac, 1965). Whether this is due to entry through the stomatal aperture or by greater permeability of the cuticle on or near the guard cells is still not known. Although it has been shown that pure water (and presumably other liquids of high surface tension) cannot enter stomatal apertures (Schonherr and Bukovac, 1972), there is convincing evidence that liquids of reduced surface tension can do so (Dybing and Currier, 1959 and 1961; Green and Bukovac, 1974;

Schonherr and Bukovac, 1972). In addition Sands and Bachelard (1973) showed good correlation between stomatal aperture and uptake of picloram in Eucalyptus. Other workers, however, suggest that whilst uptake may or may not be correlated with stomatal frequency, it continues at the same rate when the stomatal aperture is closed (Weaver and De Rose, 1946; Green and Bukovac, 1977). It is now generally believed that whilst limited stomatal aperture penetration may occur, the increased permeability of the guard cells, and possibly of subsidiary cells also, is of greater importance.

Guard cell cuticles have been shown to be major sites of adsorption of ions (Yamada, Rasmussen, Bukovac and Wittwer, 1966) and regions of preferential permeability to tritiated water (Maercker, 1965). Also the technique of Franke (1961), which involves the treatment of plant material with Gilson's reagent (a histological fixative containing mercuric salts), shows sites in the cuticle which are interpreted as interfibrillar spaces in the epidermal cell walls, and termed 'ectodesmata'. Franke points out that these features are associated with guard cells, although they have not been established by any other method.

Besides stomata, trichomes have been reported as major sites of uptake of fluorescent dyes (Butterfass, 1956; Hall, Morton and Wharrie, 1975) though in neither case is it clear how much of the chemical that enters the trichome can then move further into the leaf. Finally, both the anticlinal walls of epidermal cells and regions of the epidermis over veins, seem also to be preferential sites of penetration (Dybing and Currier, 1961), of ion binding (Yamada et al, 1966) and of ectodesmata (Franke, 1967, 1969).

3. MATERIALS AND METHODS

3.1 Plant Culture

Two plant species were used; radish (Raphanus sativus L.) was chosen as a dicotyledonous species showing a high degree of susceptibility to broad-spectrum growth regulator herbicides such as MCPA. In this respect the radish resembles the weed species wild radish (Raphanus raphanistrum L.) but is much easier to grow. To avoid any problems of assessment due to differential growth of the shoot and swollen storage root, a radish c.v. Long Black Spanish was chosen, which does not produce a large root. The second species was wild oat (Avena fatua L.), a monocotyledonous weed which is a widespread problem in Britain, particularly in cereals.

Both plant species were grown in sandy loam topsoil from Begbroke Hill Farm (Begbroke North field) which was supplemented with Vitax Q4, an NPK fertilizer, at 5 grams per Kilogram of soil. The plants were grown in 9 cm plastic pots and watered daily from above, but avoiding wetting the foliage of plants which had been treated with herbicide. Wild oats, of which the percentage germination was low, were germinated before sowing to ensure that there was one plant per pot; radish seeds were sown at 2-3 seeds per pot and thinned to one plant at the cotyledon stage. During winter months (from mid-October to early April) all plants were grown in a glasshouse in which day-length was maintained at a constant 14 hours by use of sodium lighting and temperature was not allowed to fall below 8°C during daytime and 4°C at night. Daytime maximum temperature, however, varied considerably according to solar radiation.

During summer months wild oats were grown outside so that growth and surface characteristics would be nearer to those of field-grown plants.

To minimise damage from frit fly (Oscinis frit), 0.15g of phorate insecticide granules was added to each pot. The granules were placed in a layer 1 cm below seed level using a dispenser made for that purpose, thereby avoiding damage to the plants which occurred when the granules were placed at seed level. All outdoor-grown wild oats were kept in the glasshouse after herbicide treatment to avoid the deposit being washed off by rain. Radishes were grown in the glasshouse all year round because plants grown outdoors suffered unacceptable leaf damage from phytophagous insects; it proved impossible to achieve control of the damaging insects with a light application of DDT. It was considered more important, however, that wild oats should be grown outside whenever possible, as indoor plants developed extra long leaf sheaths and showed delayed tiller production, features which might well affect response to herbicides. Full records of conditions both in glasshouses and outside have been kept at W.R.O.

Before treatment plants were selected for uniformity as judged by the number of leaves and (where appropriate) tillers. A sample of plants was weighed after noting the growth stage in most experiments, and a representative individual was recorded using a Xerographic copier. With wild oat growth stage was recorded using the code described by Tottman (1976). Thus a leaf is defined as such by the visibility of the next emerging leaf. Incompletely expanded leaves were estimated as fractions of the size of the youngest fully expanded leaf.

3.2 Herbicides and Formulations

The influence of form of deposit on performance was assessed for four herbicides. These were MCPA (4-chloro-2-methylphenoxyacetic acid), and difenzoquat (1,2-dimethyl-3,5-diphenyl-1H-pyrazolium cation), paraquat (1,1'-dimethyl-4,4'-bipyridilium cation) and glyphosate (N-(phosphonomethyl) glycine). Difenzoquat was obtained as the technical crystalline methyl sulphate salt containing 66.4% w/w of cation. The other three herbicides were obtained as aqueous concentrate solutions without surfactants and other additives of commercial formulations, so that these could be added in varying amounts in experiments. These concentrates contained the equivalent of 400 gl^{-1} of MCPA acid as the potassium salt, 200$g.l^{-1}$ of paraquat cation as the dichloride and the equivalent of 480$g.l^{-1}$ of glyphosate acid as the isopropylamine salt.

Concentrations of the herbicides applied in experiments are given in Table 3.2.1, but not all concentrations were used in all experiments. In most cases the concentration needed to apply the normal recommended rate at a volume rate of 201.ha^{-1} was used. Subsequent mention of concentration in this thesis will always refer to active ingredient, that is cation of difenzoquat and paraquat and acid equivalent of MCPA and glyphosate. 'Agral' surfactant (90% alkyl phenol ethylene oxide condensate) was added to the herbicide solutions at 0.1% v/v for MCPA, paraquat and glyphosate and 0.5% v/v for difenzoquat in all experiments except where surfactant concentration was itself under investigation.

Table 3.2.1 Herbicide concentrations used in experiments ($g.l^{-1}$ active ingredient)

Herbicide	Volume rate ($l.ha^{-1}$) to apply recommended dose*					
	5	10	20	40	80	160
MCPA	280	140	70	35	-	-
difenzoquat	200	100	50	25	-	-
paraquat	112	56	28	14	-	-
glyphosate	289	144	72	36	18	9

* Since recommended doses often vary with product, formulation and use, the following were taken as normal recommended doses:

MCPA - 1.4 kg ae/ha

difenzoquat - 1.0 kg ai/ha

paraquat - 0.56 kg ai/ha

glyphosate - 1.44 kg ai/ha.

3.3 Application of herbicide and other experimental solutions

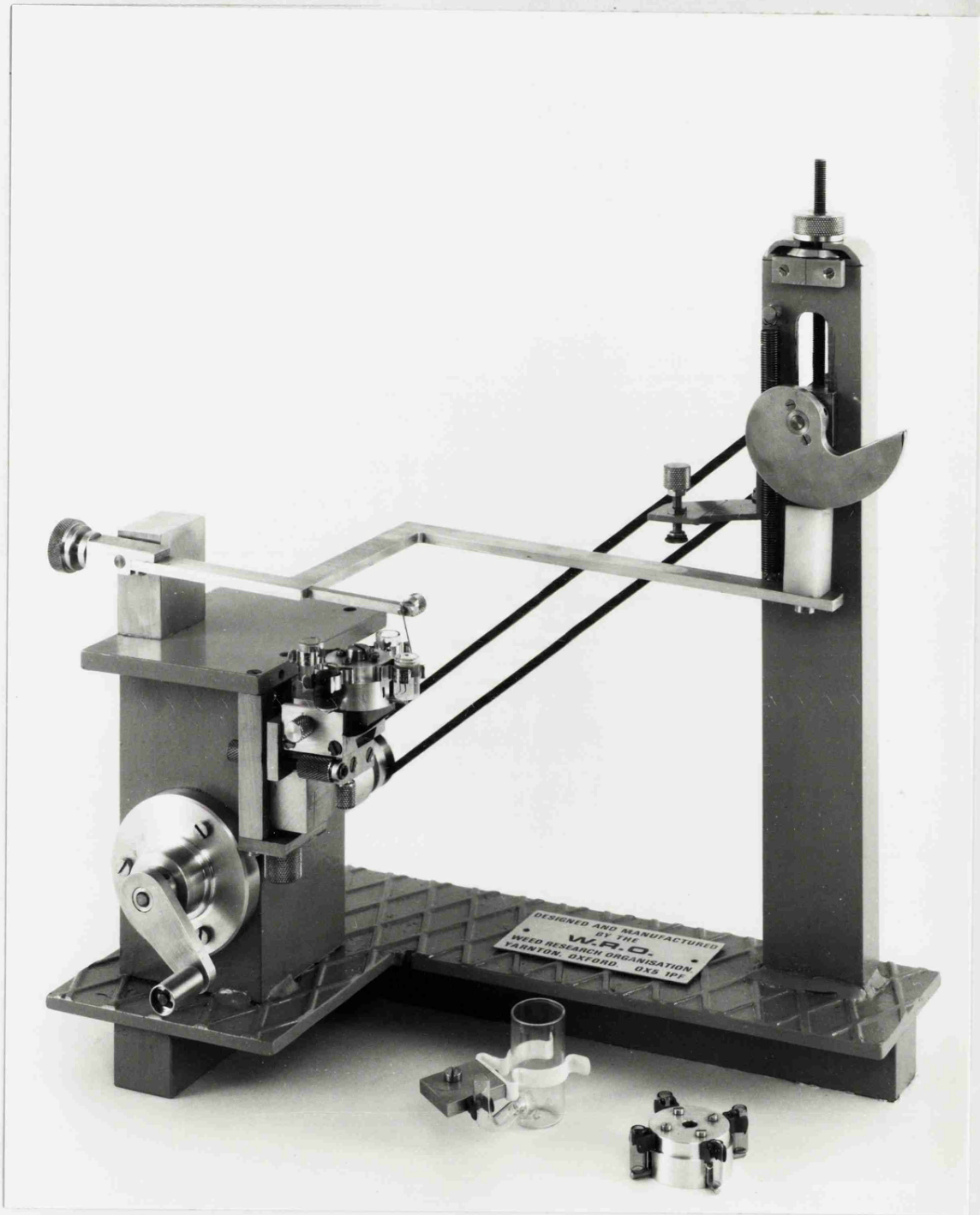
In most cases single drops of experimental solutions were applied with a device specifically designed and built for this purpose. Drops larger than 500 μm diameter (0.0625 μl) were applied using a microsyringe, either of the micrometer-operated type (the 'Agla' microsyringe) or chromatography syringes such as the 'Hamilton'. In retention studies the plants were sprayed in a laboratory spray cabinet.

3.3.1 The single drop applicator

This device was based on an earlier version described by Merritt and Drinkwater (1977). In operation a needle is partly immersed in the sample liquid before being rapidly withdrawn. By this action liquid adhering to the needle forms a ligament at the needle tip from which a single drop detaches. The drop travels upward a short distance on a parabolic trajectory before falling, when it can be collected on a target a few millimetres in front of the needle. The movement of the needle is derived from the turning of a snail cam which presses down the bar on which the needle is mounted, releasing it to return by spring action as the heel of the cam is passed.

Whilst the basic operating principle was the same in both machines, the new version which was built for this work incorporated several modifications, which are illustrated in Plate 1. The design was altered by moving the position of the cam to a point effectively twice the distance from the pivot than that of the needle (in the first version the cam was between the needle and the pivot). This change gave more precise control over drop size. To accommodate this change there had to be a double right-angled step in the bar so that drops did not hit the bar, and also to allow manipulation of plants below the needle. To keep the cranking handle in a convenient position the cam

Plate 3.1 The single drop Applicator.


DESIGNED AND MANUFACTURED
BY THE
W.R.O.
WEED RESEARCH ORGANISATION
YARNTON, OXFORD. OX5 1PF

was turned by means of a rubber drive belt. The experimental liquid is held in one of three interchangeable containers of volumes 25, 1 and 0.5 cm^3. The two smaller sizes are made from cut down gas chromatography vials, and mounted on rotatable blocks with four containers on each. Thus four solutions or drop sizes can be calibrated at one time. The sample holders are mounted on a three-directional adjustable stage. Horizontal movement in two directions makes it possible to centre the vials under the needle. Vertical movement alters the depth to which the needle dips into the sample liquid, and hence the amount of liquid adhering to the needle on withdrawal, which determines the size of drop produced. As a further modification (not shown in Pate 1) a protractor scale was mounted on the cam face and a mark made on the nylon cam-follower. This enabled the operator to turn the cam by hand until the needle tip just touches the meniscus of the sample liquid, providing a base setting in degrees of cam turn from the heel, to which the liquid level can be reset, thus avoiding the need for frequent re-calibration of the drop size.

Calibration of the drop size was achieved as described by Merritt and Drinkwater (1977). Drops of aqueous solutions of herbicides produced by the single drop applicator were caught in a cell containing silicone fluid, specific gravity 0.97 and viscosity 10,000 cs over which a thin film of paraffin had been placed. The drops then sink very slowly in the silicone fluid in a spherical form so that they can be viewed using a microscope with a long-focusing objective and measured with a micrometer eyepiece.

Data obtained show that variability of deposits produced is greater using the single drop applicator than with microsyringes, though still acceptably low; typical values for the coefficient of variation

of volume with deposits of eight 300 μm drops being around 5-10% whilst those for microsyringe applications of five 0.2 μl drops were 2.5-5%. Particular precautions taken which helped reduce variability of applications included frequent cleaning of the needle and topping up of the sample liquid holder using a syringe so that the meniscus shape remained constant.

3.3.2 The spray cabinet

In experiments to determine the retention of spray on plants the spraying was performed in a large laboratory cabinet. This cabinet has a trolley propelled by an electric motor via a winch at uniform speeds up to 1.7 ms^{-1}. Plants to be sprayed are placed in a chamber below the trolley on a table which can be varied in height. The trolley is designed to carry conventional hydraulic nozzles, operated by compressed air, or a controlled drop application head comprising five spinning discs and employing a battery-driven electric motor for disc drive and a low pressure liquid flow system. The spinning disc head is capable of producing drop sizes of 150-350 μm diameter in relatively uniform sizes.

3.4 Measurement of spray retention on plants

Plants grown outdoors were sprayed in the cabinet described in Section 3.3.2. Only outdoor plants were used since preliminary work had shown that retention by glasshouse-grown plants differed from that of outdoor plants as did the general morphology of the plants.

Herbicides were omitted from the spray solutions but surfactant was included at the recommended concentrations to maintain the surface tension at the normal level; the presence of herbicide made little or no difference to the liquid physical characteristics but caused problems with the assay technique. Fluorescein sodium salt was included in all spray solutions at 1.0 $g.l^{-1}$ as a tracer for measurement of both retention of spray on plant surfaces and the ground spray volume rate. The latter was measured by placing petri dishes between the plants and washing out the collected spray with distilled water, the resultant solution being placed in a Turner 110 filter fluorimeter and its fluorescence compared to a range of standards.

To measure retention by plants, the plants were cut at soil level then divided into the required sections. Each plant section was placed in a polythene bag and a solution of 0.1 ml. l^{-1} Agral added in sufficient quantity to ensure a thorough washing of the plant material to remove the retained dye. The bag with sample and washing solution was shaken vigorously for 30 seconds, then the plant washings poured off. A sample of 10 ml was removed and 0.5 ml of 0.1 M sodium hydroxide added; the addition of sodium hydroxide had previously been found necessary to counteract a fluorescence quenching effect which occurs when fluorescent dyes are present in plant washings. Preliminary studies had shown the optimal amount of sodium hydroxide to be added. The samples were then placed in a Jobin Yvon JY3 spectrofluorimeter and

fluorescence measured at 514 nm using an excitation wavelength of 493 nm and a slitwidth of 4 nm. Standards made from the spray solution using an Agla microsyringe were similarly assayed and unsprayed plants were washed and assayed for any background fluorescence, which was normally found to be zero with this technique.

The plant material, after washing, was placed in 'Kraft' paper bags and oven-dried at 95°C for 48 hours for dry weight determination.

3.5 Measurement of the rate of evaporation of drops

In an attempt to measure the rate of evaporation of spray drops an indicator was sought. Placement of large drops on a delicate balance weighing to 0.1 mg was feasible, but of no use for drops of the required size of 100 to 500 μm. Measurement of the evolution of water vapour was considered impractical at these drop sizes with available equipment for humidity measurement and would anyway be very difficult where drops were placed on plant surfaces which were themselves producing more water vapour by transpiration than the evaporating drops.

Thus a chemical indicator was sought, cobaltous chloride, a well known indicator of water, was found to require very high concentrations to give visible colour changes, and it was felt that such concentrations would greatly affect evaporation rate by the colligative elevation of boiling point.

Finally sodium fluorescein was tried and found very suitable. Drops of a 0.5 $g.l^{-1}$ solution were easily seen in a darkened chamber, when illuminated with an ultra-violet lamp, at the required drop sizes and well below these. These drops, when placed on a non-absorbent surface such as glass or on leaf surfaces, fluoresced as long as there remained water in the drop. However when sodium fluorescein dries out on such a non-absorbent surface it crystallizes, and when this occurs fluorescence ceases. This general feature is reliable unless drying takes place on a surface on which fluorescein ions adsorb or are otherwise physically kept in an amorphous state, such as when drying occurs to drops absorbed by filter paper. Thus by this technique the end point of evaporation was found to be relatively simply assessed. Drops of 0.5 $g.l^{-1}$ fluorescein solution were evaporated in a darkened chamber containing an ultra-violet lamp and the time to evaporation taken with

a stopwatch. Humidity and air temperature in the chamber could be varied by conducting the experiments in different controlled environment rooms.

3.6 Assessment of biological effect

The herbicides used in this investigation are normally employed to reduce the growth of or kill weed plants. A range of doses below that which causes maximum reduction in growth, or death, is generally used to facilitate comparisons between different treatments. The most widely used method of assessing the growth of plants after herbicide treatment is measurement of the fresh or dry weight (or both) of the plant, and for reasons of ease and speed the shoot alone (or plant parts above soil level) is often taken for this purpose. Thus in experiments described in Section 4.3, the effects of form of deposit on herbicide performance were usually assessed in terms of fresh or dry weight of shoot determined some time after treatment. Fresh weights were assessed by cutting the shoot at soil level and immediately weighing on a top-pan balance accurate to 1 mg, having watered the plants one or two hours before assessment to ensure that they were fully turgid. For dry weight determination the plant material was then placed in 'Kraft' paper bags and left in an oven at 95°C for 48 hours. The dried material was then weighed to either 1.0 or 0.1 mg according to the general sample size in the experiment.

Since the experiments varied considerably in nature no fixed interval to assessment was chosen. Instead this was decided according to the individual experiment, and varied depending on factors such as rate of growth under the conditions prevailing during the experiment and according to the size of plants at treatment. Generally such a decision can be made satisfactorily only when the response of a particular plant to a herbicide with time is known, and preliminary experiments were therefore conducted to help with this decision. In practice assessment times were similar for a given herbicide and weed.

3.7 Measurement of penetration and movement of ^{14}C-labelled difenzoquat

Difenzoquat labelled with ^{14}C at the 3 carbon position on the pyrazolium ring was obtained. A solution of 200 $g.l^{-1}$ unlabelled difenzoquat containing 0.5% Agral was prepared. To 0.5 ml of this solution was added 60 µl of a stock solution of labelled difenzoquat. The stock solution contained 7.2 $\mu g.\mu l^{-1}$ difenzoquat (specific activity 13 $\mu Ci.mg^{-1}$) so that the final solution contained approximately 11.2 x 10^{-3} $\mu Ci.\mu l^{-1}$. The other solutions used were made from this solution by diluting with the appropriate amount of Agral solution.

The labelled solutions were applied either by microsyringe or single drop applicator. The test plants were then harvested after various time intervals and separated into samples for the determination of the level of radioactivity in the various plant parts. Previous work has already shown that no significant alteration in the difenzoquat cation by metabolism or breakdown occurs over the time scale used in these experiments (Sharma et al, 1976).

Samples prepared from the test plants were as follows. First the treated leaf or area of leaf was washed with 10 ml of 0.5% Agral solution to remove any difenzoquat remaining as a surface deposit. Plants treated and immediately washed in this way were used for determination of the total application rate and its variability. A 1.0 ml aliquot of the plant washings was then added to 10 ml of scintillant containing 5.5 $g.l^{-1}$ of PPO and 0.1 $g.l^{-1}$ of POPOP in 2 : 1 Toluene: Triton x-100. Radioactivity was assayed in a Packard Tri-carb liquid scintillation spectrometer.

The plant was then cut into sections. Small portions of leaf were solubilized in "Soluene" and bleached with a saturated solution of

benzoyl peroxide in toluene before being assayed in acidified scintillant prepared by adding 1 part of 0.5 M HCL to 9 parts of the Triton x-100 scintillant cocktail mentioned above. Larger plant parts, including roots, were oxidised in an oxygen train type oxidiser (the roots having been washed to remove soil particles and blotted dry). The carbon dioxide produced during oxidation was trapped in "Reich" scintillant, 1 litre of which contained 400 ml toluene, 330 ml phenylethylamine, 220 ml methanol, 7 g butyl-PDB and 50 ml water.

3.8 Fluorescence Microscopy

3.8.1 The site of uptake of fluorescent dyes

Observations on the site of uptake of fluorescent dyes were made in a manner based on earlier studies by Dybing and Currier (1959, 1961).

Test solutions containing one or more of a range of fluorescent dyes were applied to various plant surfaces either as large drops from a microsyringe (0.2-2.0 µl) or as relatively small drops using the single drop applicator, with drop diameters of 100-400 µm (volumes of 0.000524 to 0.0335 µl). After various times surface deposits were removed by washing the leaves with running water either from a tap or from a wash bottle. The leaves were then immediately mounted on a microscope slide using double-sided adhesive tape, taking care to avoid handling the treated portion of the leaf.

The location of fluorescent dye remaining in the leaf was studied using a Zeiss universal microscope. This was fitted with a high pressure 400 W mercury vapour lamp and an epi-condenser (III RS) for incident fluorescence work. The condenser contains filter combinations for work at specific narrow bands of excitation and fluorescence wavelengths. Objectives used included a variable x1.5-5.0 magnification lens and a x10 planachromat lens. Photographs were taken using a 35 mm Zeiss camera back and Kodak 'Ektachrome 200' colour transparency film rated at 800 A.S.A. Exposure times were of the order 30-120 seconds.

3.8.2 Measurement of drop spreading

In order to help with interpretation of experiments on the effects of form of deposit on herbicide performance, drop spreading

was investigated. Previous workers have used measures of contact angles of liquid drops on leaf surfaces to give comparative values of spreading (See Section 2.3.2). However the techniques used for measuring contact angles generally necessitate the use of large drops which are made to roll down inclined leaf surfaces. Since it is possible that the degree of spread is influenced by the size of drop used a method was sought of measuring spread of drops in sizes relevant to sprays and the experiments in this study. To do this a fluorescent dye, Tinopal CBS, was included at a concentration of 5.0 $g.l^{-1}$ in test solutions containing 0.1 or 0.5% Agral. This dye produced a deposit which is still fluorescent after drops have dried. Thus it was possible to photograph the dried drop deposits on leaf surfaces using the fluorescence miscroscope as described in the preceding section. The drop deposits were photographed on Ilford FP4 black and white film. Since the deposits were highly irregular in shape, the areas of the drop stains had to be measured. This was achieved by means of a Quantimet image analyser, which recorded the areas of the drop images on the negatives.

3.9 Analysis and presentation of results

Experiments on biological response to varying form of deposit, described in Section 4.3, were all of randomised block design. Treatments were arranged factorially with normally only one or two main factors in addition to variable dose. This meant that analysis of variance of the results was a straightforward process with interactions between factors kept to a minimum. The results of these experiments have been presented in the form of graphs or histograms using data converted to a percentage of the untreated control, with comments on the analysis of variance in the text at the appropriate place. Experiments in Section 4.4 on ^{14}C tracing of difenzoquat are similarly analysed and presented except that the results were first converted to a percentage of the total counts per minute of radioactivity applied, then subjected to a standard angular transformation for analysis of variance. Different sections of the plants (such as treated leaf and rest of shoot) were analysed separately since values often differed widely. In all graphs and histograms bar lines represent the standard error.

Results in other sections were usually analysed by calculating standard errors and employing t-tests for comparisons between means.

4. RESULTS AND DISCUSSION

4.1 Experiments on spray retention

A change in the method of herbicide application is likely to bring about changes in the amount of spray liquid retained by the target plants, and also in the distribution of that spray liquid around the various surfaces of the plants (See Section 2.3.2). A knowledge of such changes may be necessary to interpret experiments on the effect of position of deposit on herbicide performance. For example, if a certain position on a plant is more effective as a site of treatment this may only be of importance if that site represents a significant target for the retention of spray drops.

The experiments described in this section were designed to investigate the distribution of retained spray on the surfaces of wild oat, radish and barley.

4.1.1 Positional retention on wild oats

Three experiments were conducted, the first two using three-leaf wild oat plants, and the third plants with four leaves and four tillers.

The first two experiments differed only in a slight variation in plant size, mean dry weights of foliage being 19.6 and 28.1 mg per plant respectively. In both these experiments two batches of plants were sprayed in the laboratory spray cabinet. One batch was sprayed using the CDA unit, with a mean drop size of 250 μm diameter and a measured volume rate of 22.5 and 22.1 $l.ha^{-1}$ for experiments 1 and 2 respectively. A second batch of plants in each experiment was sprayed with conventional flat fan hydraulic nozzles ('Teejet' 8004) at 2.1 bars pressure giving volume rates of 193 and 234 $l\ ha^{-1}$ in

experiments 1 and 2 respectively. The spray solution was 0.5% v/v 'Agral', the concentration recommended for the application of difenzoquat, with 1.0 gl^{-1} fluorescein (sodium salt) added for retention measurement.

The surface tension of this solution was 32.5 mNm^{-1}. In both experiments the plants were divided into four sections for determination of the position of retained spray. These were leaf 1, leaf 2, leaf 3 and the rest of the plant, this latter section comprising the leaf sheaths of leaves 1 and 2 plus the first emerging tiller, which never exceeded 1.0 cm in length. Leaves 1 and 2 were cut at the ligule and leaf 3, which was not completely unfurled, was cut at the ligule of leaf 2.

The results are presented in Tables 4.1.1-4.1.4. In both experiments the greatest proportion of spray retained with the CDA treatment was on the second leaf (53 and 45% of the total in experiments 1 and 2 respectively). Leaf 3 was relatively larger compared to leaf 2 in the second experiment, in which slightly larger plants were used, and consequently its proportion of the retained spray increased slightly. With the conventional application leaf 3 retained a greater proportion than with the CDA application, and in experiment 2 this leaf retained more than leaf 2. Since leaf 3 is more upright than leaves 1 and 2 it is likely to be less effective as a target to vertically falling drops such as those from controlled drop application. In contrast the hydraulic nozzle spray has a considerable proportion of its drops moving in non-vertical trajectories, both because of the angular nature of the spray fan and because air movement accompanying spray formation is likely to impart turbulence into the trajectories of the smaller drops. Furthermore, the smaller drops may be more

Table 4.1.1 The distribution of spray retained on wild oats (growth stage : 13) sprayed with 0.5% 'Agral' at

a) 22.5 $l.ha^{-1}$ using 250 µm diameter drops (CDA) or

b) 193 $l.ha^{-1}$ with conventional hydraulic nozzles (CON.)

mg = Dry weight in mg.

µl = Volume of spray retained.

SE = Standard error of ul retained.

% = % of total plant retention on each section.

a) CDA

Plant Section	mg	µl	SE	%
leaf 1	4.22	0.184	0.0161	26
leaf 2	7.11	0.373	0.0306	53
leaf 3	4.61	0.113	0.0154	16
Rest of plant	3.28	0.035	0.0075	5
Total	19.22	0.705	-	100

b) CON

Plant Section	mg	µl	SE	%
leaf 1	4.54	0.959	0.0637	23
leaf 2	7.56	1.860	0.179	45
leaf 3	5.05	0.803	0.0869	19
Rest of plant	2.74	0.529	0.0493	13
Total	19.89	4.151	-	100

Table 4.1.2 The distribution of spray retained on wild oats (growth stage : 13) sprayed with 0.5% 'Agral' at 22.5 $l.ha^{-1}$ using 250 µm diameter drops (CDA) or 234 $l.ha^{-1}$ with conventional hydraulic nozzles (CON.)

a) CDA

Plant section	mg	µl	SE	% of total
leaf 1	6.10	0.21	0.019	26
leaf 2	8.34	0.36	0.030	45
leaf 3	7.82	0.16	0.017	20
Rest of plant	6.38	0.07	0.014	9
Total	28.64	0.80	-	100.00

b) CON.

Plant section	mg	µl	SE	% of total
leaf 1	6.15	0.96	0.083	17
leaf 2	8.54	1.34	0.138	24
leaf 3	7.15	2.07	0.172	37
Rest of plant	5.62	1.17	0.094	21
Total	27.46	5.54	-	100.00

Table 4.1.3 Calculated µg a.i. retained assuming an applied dose of 1 kg (a.i.). ha^{-1} on wild oats (growth stage : 13) sprayed with 0.5% 'Agral' at 22.5 $l.ha^{-1}$ using 250 µm diameter drops (CDA) or 193 $l.ha^{-1}$ with conventional hydraulic nozzles (CON.)

Treatment		leaf 1	leaf 2	leaf 3	Rest	Total
CDA	µg/section	8.17	16.58	5.02	1.56	31.33
	SE	0.715	1.360	0.684	0.333	-
CON	µg/section	4.97	9.64	4.16	2.74	21.50
	SE	0.330	0.927	0.450	0.255	-
CDA	µg/mg(s)	2.16	2.37	1.48	0.60	6.61
	SE	0.186	0.119	0.199	0.179	-
CON	µg/mg(s)	1.19	1.44	1.16	1.54	5.33
	SE	0.085	0.211	0.166	0.127	-
CDA	µg/mg(t)	0.462	0.929	0.255	0.092	1.738
	SE	0.031	0.062	0.029	0.025	-
CON	µg/mg(t)	0.266	0.311	0.159	0.142	0.878
	SE	0.019	0.057	0.029	0.013	-

Table 4.1.4 Calculated µg a.i. retained assuming an applied dose of 1 kg (a.i.) ha^{-1} on wild oats (growth stage : 13) sprayed with 0.5% 'Agral' at 22.5 $l.ha^{-1}$ using 250 µm drops (CDA) or 234 $l.ha^{-1}$ with conventional hydraulic nozzles (CON.)

Treatment		leaf 1	leaf 2	leaf 3	Rest	Total
CDA	µg/section	9.50	16.29	7.24	3.16	36.19
	SE	0.878	1.339	0.756	0.634	-
CON	µg/section	4.10	5.72	8.84	5.00	23.66
	SE	0.355	0.589	0.734	0.401	-
CDA	µg/mg(s)	1.54	1.90	0.996	0.543	4.98
	SE	0.120	0.115	0.062	0.070	-
CON	µg/mg(s)	0.705	0.709	1.286	0.987	3.69
	SE	0.061	0.071	0.015	0.171	-
CDA	µg/mg(t)	0.353	0.593	0.262	0.109	1.32
	SE	0.031	0.045	0.020	0.016	-
CON	µg/mg(t)	0.148	0.205	0.324	0.194	0.87
	SE	0.012	0.020	0.030	0.023	-

readily retained on the young vertical leaf than larger drops, as is suggested by observations of plants sprayed with hydraulic nozzles using fluorescent dyes in the spray and studied under ultra-violet light. Even greater differences between the two spray methods occurred on the fourth plant section comprising the leaf sheaths. Here the conventional spray produced $2\frac{1}{2}$ times the retention of that from the CDA spray. This may be important to the activity of a herbicide like difenzoquat (Caseley and Coupland, 1980).

For ease of comparison between the two spray methods, figures of dose of active ingredient which would be retained assuming a dose of 1.0 Kg a.i. ha^{-1} were calculated (Tables 4.1.3 and 4.1.4). Three separate values were calculated for each treatment and plant section. Firstly the expected amount of herbicide in ug per section was calculated, secondly a figure for ug retained divided by the dry weight of the section (labelled $\mu g/mg(s)$) and thirdly the amount in ug retained divided by the total dry weight for the whole plant (labelled $\mu g/mg(t)$). The $\mu g/mg(s)$ produces a figure showing the efficiency of a given section as a retaining surface relative to its own size, whilst the $\mu g/mg(t)$ shows the retention efficiency relative to the whole plant size, and therefore probably more closely related to the biological importance of the retained spray, assuming the herbicide toxicity is related to amount of plant tissue.

Calculation of the amount of herbicide retained for a standard dose permits more meaningful comparison between the different spraying methods and volume rates. For example, although the wild oats retained more spray liquid by conventional treatment than CDA (Tables 4.1.1 and 4.1.2), they actually retain more herbicide by CDA (Tables 4.1.3 and 4.1.4). Comparing the values for $\mu g/mg(t)$, CDA sprayed plants

retained about twice as much herbicide as conventionally sprayed plants in experiment 1 (Table 4.1.3) and $1\frac{1}{2}$ times as much in experiment 2 (Table 4.1.4). Even so, there is more herbicide retained on the leaf sheath section of the plants with the conventional application in both experiments, and in experiment 2 more herbicide also on leaf 3 with the conventional application. Nevertheless for this to be of major importance for toxicity these positions would have to be considerably more effective as sites of treatment to counteract the much larger proportions of spray retained by leaves 1 and 2 with both spray methods.

The third experiment with wild oats was conducted with larger plants, having four leaves on the main shoot with the fifth leaf just emerging, and four tillers of which two were basal tillers (emerging from below soil level) and two were axial tillers (emerging from the axils of leaves one and two). Leaf three was the youngest fully expanded leaf (ie with the ligule exposed) and the mean dry weight of the plants was 0.107 grams.

The plants were sprayed with a drop size of 300 μm using the CDA sprayhead and the volume rate was recorded as 17 l ha^{-1}. The plants were divided into seven sections for retention determination and these various sections were combined to obtain a total dry weight for each plant.

The results of this experiment are given in Table 4.1.5. The largest proportions of spray were retained by the youngest fully expanded leaf (leaf 3) and the section comprising leaf 4 plus leaf 5 (where present). These two leaves were closely followed, in quantity retained, by the first axial tiller which was quite large with these plants. Leaves 1 and 2 had much smaller proportions on them, although the calculated weights of active ingredient which would be retained are

Table 4.1.5 The distribution of retained spray on wild oats (growth stage : 14, 24; Mean dry weight = 0.107g) sprayed with 0.5% v/v 'Agral' at 17 $l.ha^{-1}$ using 300 μm diameter drops

Plant section	µl	SE	µg**	% of total	Calculated No. of drops	µg/mg(t)
leaf 1	0.20	0.014	11.8	6.7	14	0.114
leaf 2	0.34	0.020	19.8	11.3	24	0.189
leaf 3	0.66	0.036	38.6	22.1	47	0.366
leaves 4 & 5*	0.64	0.046	37.5	21.4	46	0.341
Basal tillers	0.43	0.041	25.2	14.4	31	0.231
1st axial tiller	0.57	0.041	33.8	19.3	41	0.303
leaf sheaths + 2nd axial tiller	0.14	0.013	8.4	4.8	10	0.076
Total	2.98	-	175.1	100	213	1.620

* Leaves 4 and 5 were not completely expanded and therefore were cut at the point of emergence from the sheath of leaf 3.

** Calculated µg a.i. retained assuming 1 $kg.ha.^{-1}$

greater than with the same leaves on the younger plants (See Figures 4.1.3 and 4.1.4), suggesting that some growth has occurred even after complete emergence of the leaves, or possibly that there are changes either in surface quality or leaf angle after emergence. The amount of spray retained by the leaf sheaths and the second axial tiller was small, both in proportion of the total plant retention and in relation to the plant dry weight, suggesting that the contribution of spray retained on these parts to the overall toxic response would be small, unless they proved to be considerably more active as sites of application than other plant parts.

4.1.2 Positional retention on radish

Radishes with two expanded foliar leaves were sprayed with 0.1% v/v Agral solution containing 1.0 g l^{-1} fluorescein, using either the CDA spray head or conventional hydraulic nozzles. The CDA treatment gave a volume rate of 25.5 l ha^{-1} using 250 μm diameter drops and the conventional treatment gave 212 l ha^{-1} using 'Teejet' 8004 nozzles at 2.1 bars pressure. The radish plants were divided into three sections for determination of retention, namely the cotyledons, the first pair of foliar leaves and the rest of the plant. The cotyledons and foliar leaves were cut so as to include the petioles, and the rest of plant section comprised the stem and terminal bud, the latter usually including one partially emerged foliar leaf.

The results are presented in two tables; Table 4.1.6 shows the mean dry weight, actual volume in ul of spray retained and the proportion of the total retention on each section, and Table 4.1.7 shows the weight of active ingredient that would be retained assuming a herbicide dose rate of 1.0 $Kg.ha^{-1}$. The greatest proportion of spray was retained by the foliar leaves (71 and 59% for the CDA and conven-

Table 4.1.6 The distribution of spray retained on radishes (2 foliar leaves) sprayed with 0.1% 'Agral' at
a) 22.5 $l.ha^{-1}$ using 250 µm drops (CDA) and
b) 212 $l.ha^{-1}$ with conventional hydraulic nozzles (CON)

a) CDA

Plant section	mg	µl	SE	%
Cotyledons	20.5	1.95	0.13	21
Foliar leaves	77.9	6.54	0.34	71
Rest	19.1	0.74	0.06	8
Total	117.5	9.23	-	100

b) CON

Plant section	mg	µl	SE	%
Cotyledons	21.6	16.0	1.00	22
Foliar leaves	79.1	42.5	2.32	59
Rest	15.8	13.4	1.29	19
Total	116.5	71.9	-	100

Table 4.1.7 Calculated µg a.i. retained assuming an applied dose of 1kg(a.i.) ha^{-1} on radish (2 foliar leaves) sprayed with 0.1% 'Agral' at 22.5 $l.ha^{-1}$ using 250 µm drops (CDA) and 212 $l.ha^{-1}$ with conventional hydraulic nozzles (CON.)

Treatment		Cotyledons	Foliar leaves	Rest	Total
CDA	µg/section	76.5	256.5	29.0	362.0
	SE	5.1	13.3	2.4	-
CON	µg/section	75.5	200.5	63.2	339.2
	SE	4.7	10.9	6.1	-
CDA	µg/mg(s)	3.92	3.37	1.57	8.86
	SE	0.31	0.17	0.10	-
CON	µg/mg(s)	3.58	2.55	5.09	11.22
	SE	0.19	0.11	0.54	-
CDA	µg/mg(t)	0.67	2.24	0.25	3.16
	SE	0.056	0.11	0.019	-
CON	µg/mg(t)	0.65	1.72	0.54	2.91
	SE	0.041	0.094	0.052	-

tional treatments respectively). The cotyledons retained a similar proportion with both treatments but the rest of the plant retained twice as much spray with the conventional treatment as with the CDA treatment (19 and 8% respectively). From the calculated weight of herbicide retained (Table 4.1.7) a similar picture emerged with much more retention on the stem and terminal bud by conventional spraying, whilst a greater amount of herbicide would be retained on the foliar leaves with CDA. The greatest contribution to the target area of the rest of plant section probably came from the young emerging foliar leaves, which appear generally hairy because the trichomes are very close together until the leaves have expanded significantly, and it is quite possible that the small drop fraction of the hydraulic spray is preferentially retained by these trichomes. In addition the vertical stem may present a better target to the conventional spray than CDA due to the non-vertical trajectory of some drops in the conventional spray, as was suggested in the case of the young vertical leaves of wild oat. The figures for µg/mg(s) figures show that the rest of plant section is the most efficient of the three sections as a target for the conventional spray but the least effective target for CDA.

4.1.3 Positional retention on barley

Barley was included in two experiments as an example of a crop species to indicate the effects of retention differences on selectivity between crop and weed. The barley plants, which were from one batch having $3\frac{1}{2}$ leaves on the main shoot and one tiller emerging from the axil of leaf 1, were sprayed in two experiments in conjunction with the experiments on wild oat (experiment 1) and radish, these being on consecutive days. Thus the two experiments differed only in Agral concentration, this being 0.5% in the first and 0.1% in the second. In each case the barley plants were divided into four sections

for retention measurement, namely leaf 1, leaf 2, leaf 3 and the rest of the plant, the latter comprising the leaf sheaths and the first axial tiller. The results are presented in the same way as were those for wild oat and radish, in Tables 4.1.8 - 4.1.11.

The proportional distribution of spray around the barley plants was very similar between the two experiments showing that changing the Agral concentration from 0.1% to 0.5% did not alter the position of retained spray significantly. This was true for both CDA and conventional treatments. As with wild oat the conventional treatment gave greater retention on the vertical plant parts including the leaf sheaths, but again this represented a relatively small proportion of the total plant retention. On the whole the results of these experiments show a similar pattern to those with wild oat, with the greater proportion of retained spray on the younger leaves.

The total amount of spray retained was slightly higher with 0.5% than 0.1% Agral with both spray systems, a result which agrees with other data (Clipsham, unpublished). An important contrast between the results for 0.1 and 0.5% Agral was in the relative difference in total retention between CDA and conventional spraying. Comparing the $\mu g/mg(t)$ for the two experiments the difference in total retention between CDA and conventional spraying was greater with 0.1% than 0.5% Agral. This may be of practical significance since such an increased retention by the higher surfactant concentration in combination with a low volume rate would reduce selectivity between barley and a weed species with characteristics similar to those of radish. In practice the application of low volume sprays, even by conventional equipment, would result in an increased surfactant concentration if a commercially formulated material were used containing a surfactant

Table 4.1.8 The distribution of spray retained on barley (growth stage : 13.21) sprayed with 0.5% 'Agral' at
a) 22.5 $l.ha^{-1}$ using 250 µm drops (CDA) and
b) 193 $l.ha^{-1}$ with conventional hydraulic nozzles (CON.)

a) CDA

Plant Section	mg	µl	SE	%
leaf 1	8.1	0.32	0.018	25
leaf 2	11.8	0.38	0.020	29
leaf 3	19.0	0.48	0.052	36
Rest	7.3	0.13	0.017	10
Total	46.2	1.3	-	100

b) CON

Plant section	mg	µl	SE	%
leaf 1	10.0	2.33	0.133	22
leaf 2	14.3	2.92	0.225	28
leaf 3	22.4	3.50	0.272	33
Rest	9.2	1.87	0.170	17
Total	55.9	10.62	-	100

Table 4.1.9 Calculated µg a.i. retained assuming an applied dose of 1 Kg (a.i.) ha^{-1} on barley (growth stage : 13.21) sprayed with 0.5% 'Agral' at 22.5 $l.ha^{-1}$ using 250 µm drops (CDA) and 193 $l.ha^{-1}$ with conventional hydraulic nozzles (CON.)

Treatment		leaf 1	leaf 2	leaf 3	rest	Total
CDA	µg/section	12.6	14.8	18.6	5.1	51.1
	SE	0.7	0.8	2.0	0.7	-
CON	µg/section	12.1	15.1	18.1	9.7	55.0
	SE	0.7	1.2	1.4	0.9	-
CDA	µg/mg(s)	1.65	1.26	0.98	0.71	4.60
	SE	0.14	0.05	0.10	0.08	-
CON	µg/mg(s)	1.30	1.14	0.88	1.45	4.25
	SE	0.10	0.09	0.08	0.37	-
CDA	µg/mg(t)	0.28	0.34	0.39	0.11	1.12
	SE	0.02	0.02	0.03	0.01	-
CON	µg/mg(t)	0.23	0.28	0.33	0.18	1.02
	SE	0.02	0.02	0.02	0.02	-

Table 4.1.10 The distribution of spray retained on barley (growth stage : 13.21) sprayed with 0.1% 'Agral' at
a) 22.5 $l.ha^{-1}$ using 250 µm drops (CDA) and
b) 212 $l.ha^{-1}$ with conventional hydraulic nozzles (CON.)

a) CDA

Plant section	mg	µl	SE	%
leaf 1	8.7	0.31	0.02	27
leaf 2	12.4	0.33	0.02	29
leaf 3	17.4	0.37	0.03	33
Rest	6.4	0.13	0.02	11
Total	44.9	1.14	-	100

b) CON

Plant section	mg	µl	SE	%
leaf 1	8.9	1.74	0.12	24
leaf 2	13.3	1.81	0.14	25
leaf 3	19.3	2.17	0.15	30
Rest	6.3	1.45	0.18	20
Total	47.8	7.17	-	99

Table 4.1.11 Calculated µg as retained assuming an applied dose of 1 Kg (a.i.) ha^{-1} on barley (growth stage : 13.21) sprayed with 0.1% 'Agral' at 22.5 $l.ha^{-1}$ using 250 µm drops (CDA) and 212 $l.ha^{-1}$ with conventional hydraulic nozzles (CON.)

Treatment		leaf 1	leaf 2	leaf 3	Rest	Total
CDA	µg/section	12.2	12.9	14.5	5.1	44.7
	SE	0.7	0.9	1.3	0.7	-
CON	µg/section	8.2	8.5	10.2	6.8	33.7
	SE	0.6	0.7	0.7	0.9	-
CDA	µg/section	1.41	1.06	0.86	0.78	4.11
	SE	0.09	0.07	0.06	0.08	-
CON	µg/mg(s)	0.94	0.66	0.52	1.23	3.35
	SE	0.07	0.06	0.03	0.12	-
CDA	µg/mg(t)	0.29	0.31	0.32	0.10	1.02
	SE	0.02	0.03	0.02	0.01	-
CON	µg/mg(t)	0.17	0.18	0.21	0.14	0.70
	SE	0.01	0.01	0.01	0.02	-

concentration intended for greater dilution at higher volume rates. The problem of selection of the surfactant concentration for herbicide formulations may for this reason be greater when a wide range of volume rates are recommended.

4.2 The spreading and evaporation of drops

The extent to which spray drops spread and the rate at which they subsequently evaporate are two factors which could contribute to the biological performance of a given form of herbicide spray deposit. Therefore measurements of these two quantities were taken in order to help in the interpretation of the subsequent biological experiments.

4.2.1 Drop spreading

The effects of drop size and Agral concentration on drop spreading were studied by the method described in Section 3.8.2. Agral concentrations of 0.1 and 0.5% were used and drop sizes between 119 and 370 μm diameter were applied to various leaves of 4-leaf wild oat plants. The influence of active ingredient on the spreading of drops was not studied because it was considered unlikely to be of major importance since differences in surface tension due to the addition of herbicides in the concentrations used in the biological experiments were found to be negligible (See Table 4.2.1), except with MCPA.

Drop deposits on the leaf surfaces were found to be highly irregular in shape, probably because they were affected by local variations in microtopography and wettability at the microscopic level. For example, liquid tended to spread more readily along the grooves between epidermal cells and over minor veins, and the drops were also distorted by protuberances such as trichomes. This irregular shape of drop deposits meant that area measurements had to be used rather than a linear measurement of drop diameter, as often used in calculating spread factors of drops on artificial targets with uniform surfaces.

The results of these measurements are presented in Table 4.2.2, in which an area-based spread factor facilitates comparison between

Table 4.2.1 Static surface tension measurements of solutions used in drop spreading studies and herbicide solutions

Solution	Surface Tension, mNm^{-1}
0.1% Agral	33.5
0.1% Agral + 1.0 $g.l^{-1}$ fluorescein	33.5
0.1% Agral + 70 $g.l^{-1}$ MCPA	40.5
0.1% Agral + 72 $g.l^{-1}$ glyphosate	35.6
0.1% Agral + 28 $g.l^{-1}$ paraquat	34.6
0.5% Agral	33.5
0.5% Agral + 1.0 $g.l^{-1}$ fluorescein	33.5
0.5% Agral + 25 $g.l^{-1}$ difenzoquat	34.6
0.5% Agral + 50 $g.l^{-1}$ difenzoquat	34.7
0.5% Agral + 200 $g.l^{-1}$ difenzoquat	34.8

Table 4.2.2 Areas of contact of drops on the surface of wild oat leaves and the calculated area-based spread factor (△ spread factor)

Agral Conc.(%)	Drop Size (μm)	leaf No.	No. of drops	Drop area ($\mu m^2 \times 10^5$)	△ spread factor
0.1	119	2	7	0.180	1.62
		3	6	0.147	1.32
		4	5	0.162	1.46
0.1	272	2	5	1.27	2.19
		3	6	1.44	2.48
		4	5	1.15	1.98
0.1	370	2	5	2.83	2.62
		3	5	2.34	2.17
		4	4	1.92	1.78
0.5	119	2	6	0.211	1.90
		3	6	0.180	1.62
		4	7	0.191	1.72
0.5	214	2	5	0.864	2.40
		3	5	0.683	1.90
		4	6	0.488	1.36
0.5	312	2	5	1.26	1.65
		3	6	1.26	1.65
		4	5	1.25	1.63

different drop sizes; this factor was derived from the area of the drop deposit on the leaf divided by the calculated equatorial cross-sectional area of the original drop in spherical form. These spread factors varied between 1.3 and 2.6 for 0.1% Agral and between 1.3 and 2.4 for 0.5% Agral.

There was no consistent effect of drop size on the degree of spread in the range studied. Thus the final area of the drop deposit is proportional to the original equatorial cross-sectional area of the drop, rather than its volume. This means that as drop size increases there will be an increase in the density of the active ingredient in the dried drop deposit (the term 'density' denoting the mass of a.i. per unit area).

Finally there was a suggestion from these results that spreading on leaf 2 was greater than that on leaves 3 and 4, this being the case for four out of the six comparisons made. This probably indicates a change in the surface wax component of the leaves during ageing.

4.2.2 Drop evaporation

The rate at which the volatile components of a spray solution evaporate from drops on the target surface may be expected to affect the entry of herbicide into the plant. In the present study the carrier solvent was in all cases water, and the evaporation of drops will be influenced by such factors as drop size, the degree of spreading, and environmental conditions, particularly temperature and humidity. The rate of drop evaporation was studied by the method described in Section 3.5.

Table 4.2.3 shows the evaporation times for drops of 0.1 and 0.5% Agral solutions on glass under laboratory conditions similar to

Table 4.2.3 Evaporation times for drops of aqueous Agral solutions of 0.5 and 0.1% concentrations on glass in laboratory conditions (see text for conditions)

Agral Conc. (%)	Drop Diam. (μm)	Evaporation Time(s)	SE
0.5	134	13.7	0.33
	212	33	1.56
	290	48	3.11
	339	61	2.94
	352	67	2.67
	444	98	3.98
0.1	135	8.3	0.22
	194	19.4	0.90
	240	27	1.54
	305	40	2.53
	372	54	4.06

those under which plants were treated in the experiments on form of deposit described in Section 4.3. These conditions were 65-76% R.H. (relative humidity), 17.5-18.5°C for the 0.5% Agral and 58-65% R.H., 19-19.5°C for the 0.1% Agral. Evaporation times increased with drop size from 10 seconds for drops around 130 um diameter to 60-80 seconds for 400 μm drops.

Further measurements of evaporation times were taken in the more controlled environmental conditions of growth rooms. Here evaporation rates of drops of 0.1% Agral solution from glass were compared with those of drops applied to the surface of wild oat leaves. The results, presented in Table 4.2.4, show that the rates of evaporation of drops on leaf surfaces were much lower than those of drops applied to glass. The difference may be due to differences in the spread of drops on the two surfaces. However, measurements of the spread factor on glass (Table 4.2.5) showed that whilst spread was slightly greater on glass than on leaf surfaces (Table 4.2.2) the difference was probably not large enough to account for the much slower evaporation on leaves. More probably this was due to the locally raised humidity around the leaf surfaces resulting from transpiration.

Despite the prolonged evaporation time on leaf surfaces all drops up to 370 μm dried within 3 minutes. Thus it is likely that, other than in conditions of very high humidity, the evaporation of spray drops occurs in a relatively short time, during which it is unlikely that significant amounts of herbicide will penetrate the leaf. Uptake of herbicide from such aqueous spray drops must therefore take place from the crystalline deposit left after drying, and be due either to a maintenance of an aqueous bridge by water from the leaf or to the surfactant which remains as a low-volatility solvent on the leaf surface. This is

Table 4.2.4 Evaporation times for drops of 0.1% aqueous 'Agral' solution on glass and leaf surfaces in growth cabinet conditions

Surface	Environmental conditions RH %	Temp. °C	Drop Diam. (μm)	Evaporation time(s)	SE
Glass	62	18.5	178	14.7	0.65
			291	46	1.91
			336	63	3.72
Glass	54	19	177	12.5	0.70
			281	30	1.78
			357	51	1.50
Avena fatua leaves	54	19	157	18	1.08
			206	58	4.4
			258	73	3.8
			289	115	11.4
			366	171	8.0

Table 4.2.5 Spread factors for two 'Agral' concentrations on glass

Agral Concentration	Drop Diameter µm	Stain Diameter µm	Linear spread factor	Area spread factor
0.1%	191	397	2.08	4.30
	321	474	1.48	2.18
	413	633	1.53	2.35
0.5%	199	366	1.84	3.40
	317	514	1.62	2.63
	412	758	1.84	3.40

an important point since many previous studies on the penetration of herbicides into plants have assumed that entry is from a liquid drop and have therefore involved the use of non-drying system of constant herbicide concentration, or large drops with protracted evaporation times.

4.3 Studies on the effects of form of deposit on herbicide performance

Herbicides are used to affect the growth, survival or reproduction of the target weeds. Unless such effects are quantified there is little purpose in further study of retention, movement of herbicide in the plant or biochemical mode of action.

Laboratory experiments with herbicide sprays, using single plants in pots, cannot be regarded as representing field conditions. The lack of wind and other sources of disturbance to the spray pattern, coupled with a lack of partial shading from spray by other weeds or crop plants means that doses of herbicide retained from a spray will be higher with these laboratory-sprayed plants than those under field spraying conditions. Thus, for example, herbicide concentration must usually be reduced in laboratory experiments to maintain the normal range of biological response. Clearly this is undesirable in experiments when concentration is a variable under study. A further problem with all experiments concerned with spraying is the difficulty of separating the variables involved. For example, when volume rate is changed, several factors are varied including herbicide concentration, retention, and the positional distribution of drops around the plant surface. For these reasons the experiments described in this section were based on specific deposit patterns achieved by the application of single drops using the device described in Section 3.3.1.

The experiments are of simple randomised block design. Only a single main factor was studied in most experiments in addition to dose; a range of doses of active ingredient per plant is essential for this kind of experiment since only by comparison with the dose response can the effects of other variables be meaningfully assessed. Furthermore a range of doses increases the chance of the responses in a given exper-

iment covering the desired range in the immediately sub-lethal region.

4.3.1 Experiments with MCPA on radish

4.3.1.1 The dose response. Two experiments were carried out to study the dose response. In the first the radishes had two expanded cotyledons with the first pair of foliar leaves just emerging, the mean fresh weight of four plants being 0.64 grams. Using 300 μm diameter drops and a solution containing 70 $g.l^{-1}$ MCPA a range of doses from 0.99-63.6 μg was applied as 1-64 drops. In the second experiment the growth stage of the radishes was three fully expanded foliar leaves, and four doses were applied between 15.9 and 127.2 μg (16-128 drops). The drops were applied evenly split between the two cotyledons in the first experiment and the first pair of foliar leaves in the second experiment. Fresh weight of foliage was assessed after 19 and 25 days for experiments 1 and 2 respectively.

In both experiments a significant dose response was observed. It was noticed, however, that the results of the first experiment (cotyledon stage), shown in Figure 4.1, were more variable than those of the second experiment (Figure 4.2). A further experiment was therefore conducted in which batches of radish at the cotyledon stage were treated, then assessed at 14, 18 or 35 days after treatment. This experiment showed that the increased variability was probably due to the observed divergence of treated plants into two groups; some plants were showing regrowth by 35 days whilst others were severely stunted or dead at this date. This divergence created a bimodal distribution of plant weight which would make analysis very difficult. Also it is probable that at the later stages at which growth regulator herbicides are applied most wild radishes and similar weeds will have expanded foliar leaves.

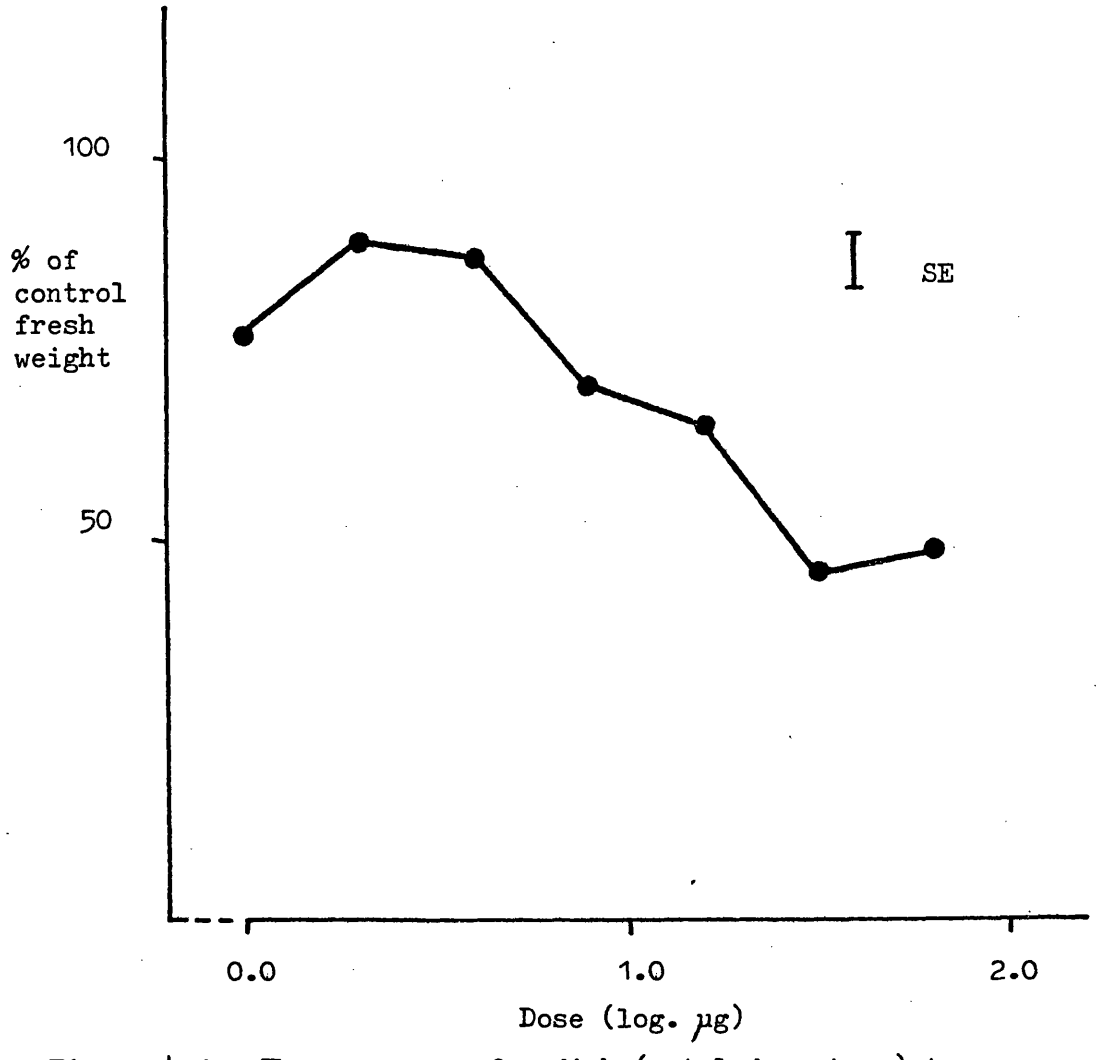


Figure 4.1: The response of radish (cotyledon stage) to a range of doses of MCPA, applied as 300µm drops using a concentration of 70 gl^{-1} a.e.

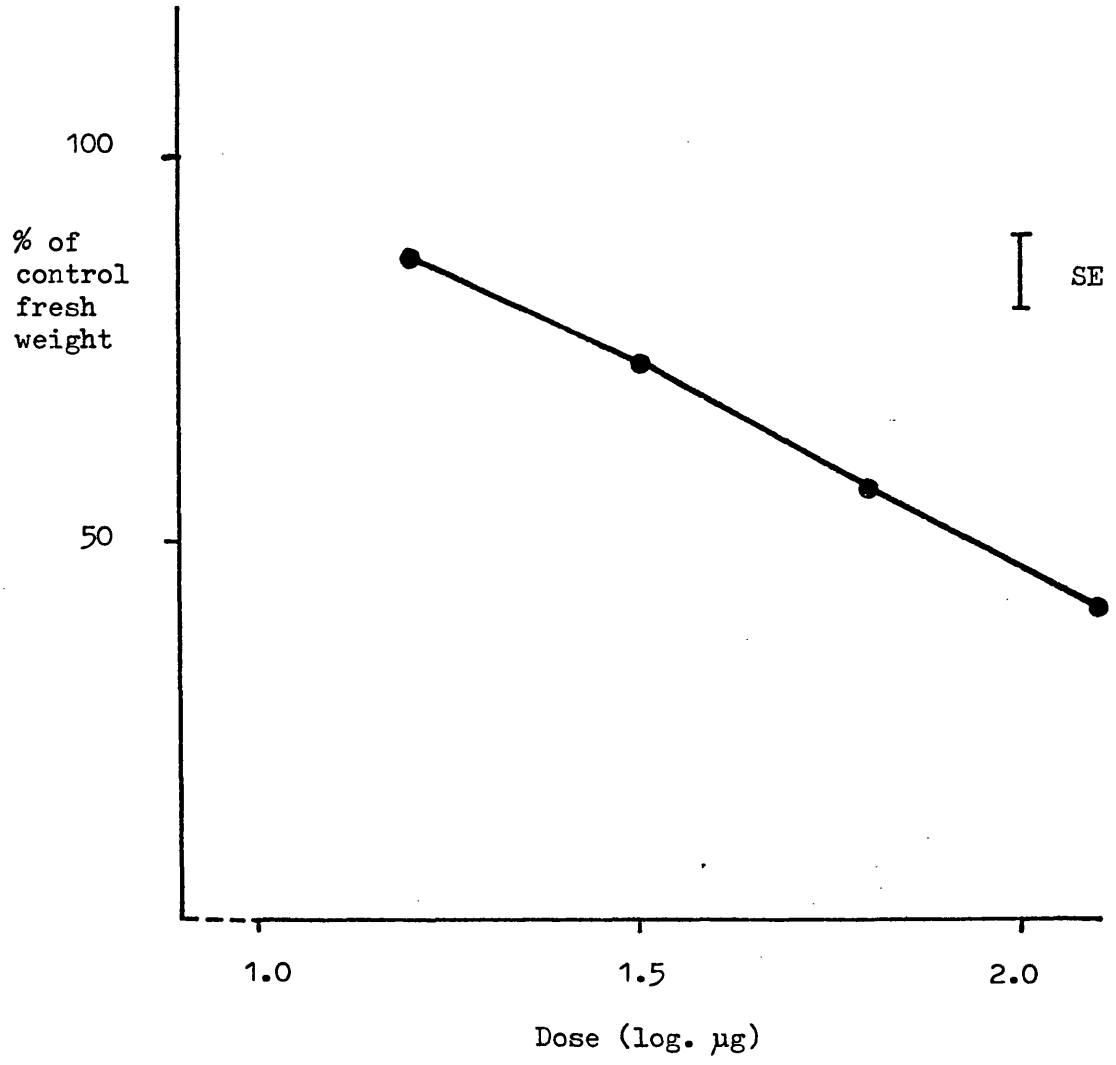


Figure 4.2: The response of radish (3 foliar leaves) to a range of doses of MCPA, applied as 300µm drops using a concentration of 70 gl^{-1} a.e.

Therefore in subsequent experiments the radishes were treated at the later growth stage with the first pair of foliar leaves expanded.

4.3.1.2 The effect of MCPA concentration. Radishes with 2-3 foliar leaves were treated on the first pair of foliar leaves with 15.9, 31.8 or 63.6 μg MCPA. These doses were applied in four concentrations, namely 35, 70, 140 and 280 $g.l^{-1}$ MCPA. The number of drops applied was varied to achieve the doses so that with the weakest solution 32-128 drops were applied (drop diameter 300 μm), whilst with the strongest solution 4-16 drops were applied. Agral was included at 0.1% v/v in all solutions. A preliminary experiment had shown that 80 drops of a solution of Agral alone at this concentration caused no significant reduction in fresh weight. The experiment comprised sixteen replicates and included untreated plants as controls; fresh weight and dry weight were assessed 16 days after treatment.

Analysis showed no difference between the results obtained with the two assessments; those for dry weight are presented in figure 4.3, showing the highly significant effect of dose ($p<0.001$), but there was no significant difference between concentrations. Thus from this experiment the concentration of MCPA does not appear to be an important factor determining the biological effect.

4.3.1.3 The effect of drop size. This experiment compared three drop sizes, namely 200, 318 and 400 μm diameter, these having volumes in the ratio 8 : 2 : 1; it was therefore possible to apply doses of 9.4, 18.8 and 37.5 μg with each drop size by varying drop number. The treatments were applied to the first pair of foliar leaves of radishes with two expanded foliar leaves and a mean fresh weight of 2.45 grams. Untreated control plants were included and there were sixteen replicates. Fresh

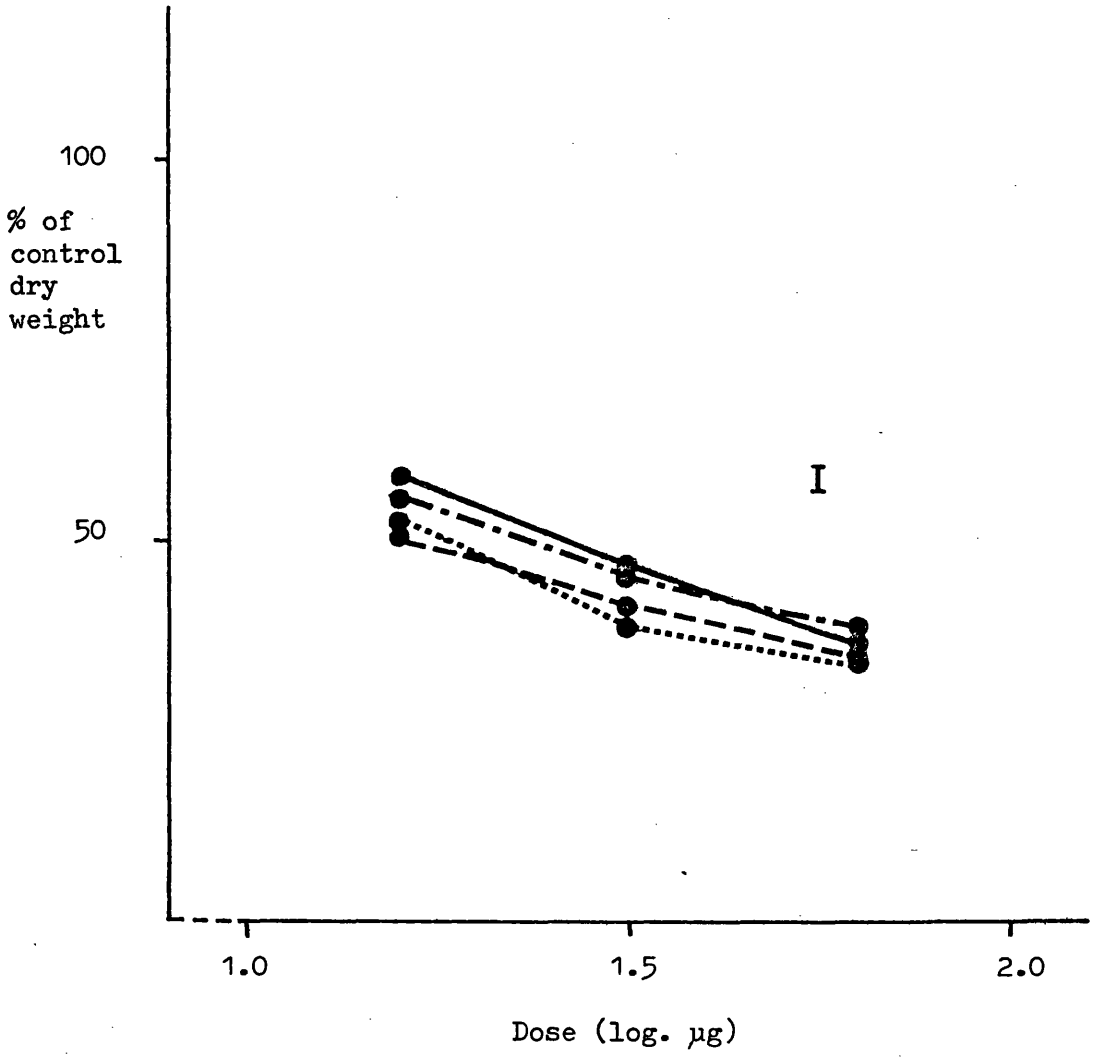


Figure 4.3: The response of radish (2-3 foliar leaves) to MCPA at three doses and four concentrations using 300 µm drops

Concentrations: 35 gl^{-1} .....

70 gl^{-1} – – –

140 gl^{-1} —·—

280 gl^{-1} ——

and dry weights were assessed 19 days after treatment.

Again there was no difference in the results of analysis for fresh and dry weights. There was a highly significant dose response ($p < 0.001$), but no significant effect due to drop size. Analysis of the ratio fresh weight : dry weight gave no significant differences at all. The results for dry weight are shown in Figure 4.4. Thus despite the eightfold difference in drop volume studied, with consequences on distribution and localisation of deposit, drop size did not influence MCPA performance.

4.3.1.4 The effect of position of deposit. The effect of varying the position of the herbicide deposit on the radish surface was studied with MCPA in two experiments. In the first experiment treatment to the first pair of foliar leaves was compared with treatment to the cotyledons and to evenly split treatment to both the cotyledons and the foliar leaves. Only two doses were applied, 15.9 and 31.8 μg, as 16 and 32 drops of MCPA solution (70 $g.l^{-1}$) using 300 μm drops. In the second experiment doses of 7.95 and 31.8 μg were applied to either the midveins of the first pair of foliar leaves or to the leaf laminae of these leaves towards the leaf margin, avoiding veins as much as possible. In both experiments untreated plants were used as controls and replicates were 20 in number in the first experiment with 10 in the second experiment. Plants had two expanded foliar leaves in both experiments. Fresh and dry weights were assessed after 15 and 20 days for the first and second experiments respectively.

Treatment of the cotyledons was inferior in performance to treatment of either the foliar leaves alone or a combination of the foliar leaves and cotyledons (Figure 4.5). There was a suggestion

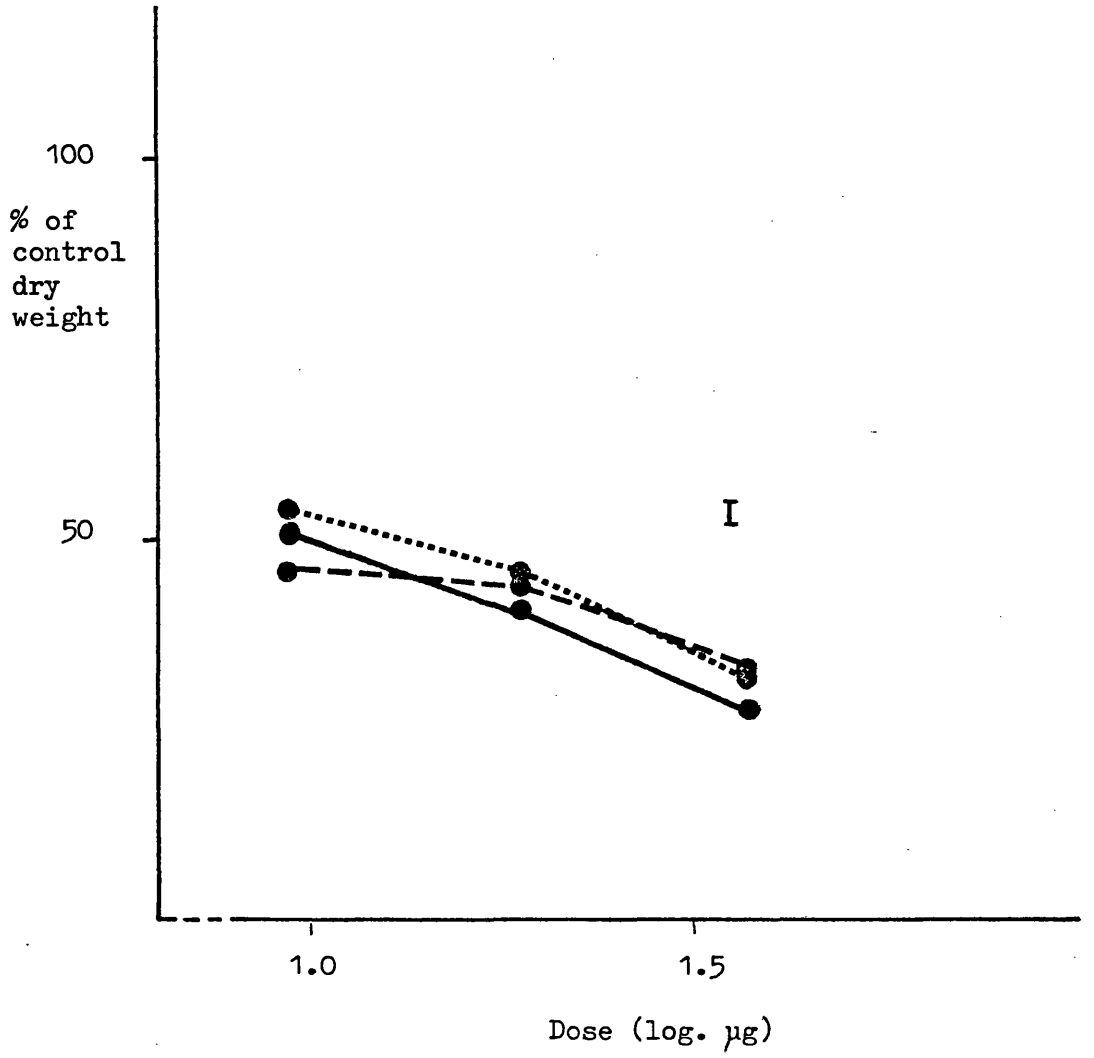


Figure 4.4: The response of radish (2 foliar leaves) to MCPA at three doses and three drop sizes using a concentration of 70gl^{-1} a.e.

Drop sizes: 200 µm .....

318 µm – – –

400 µm ——

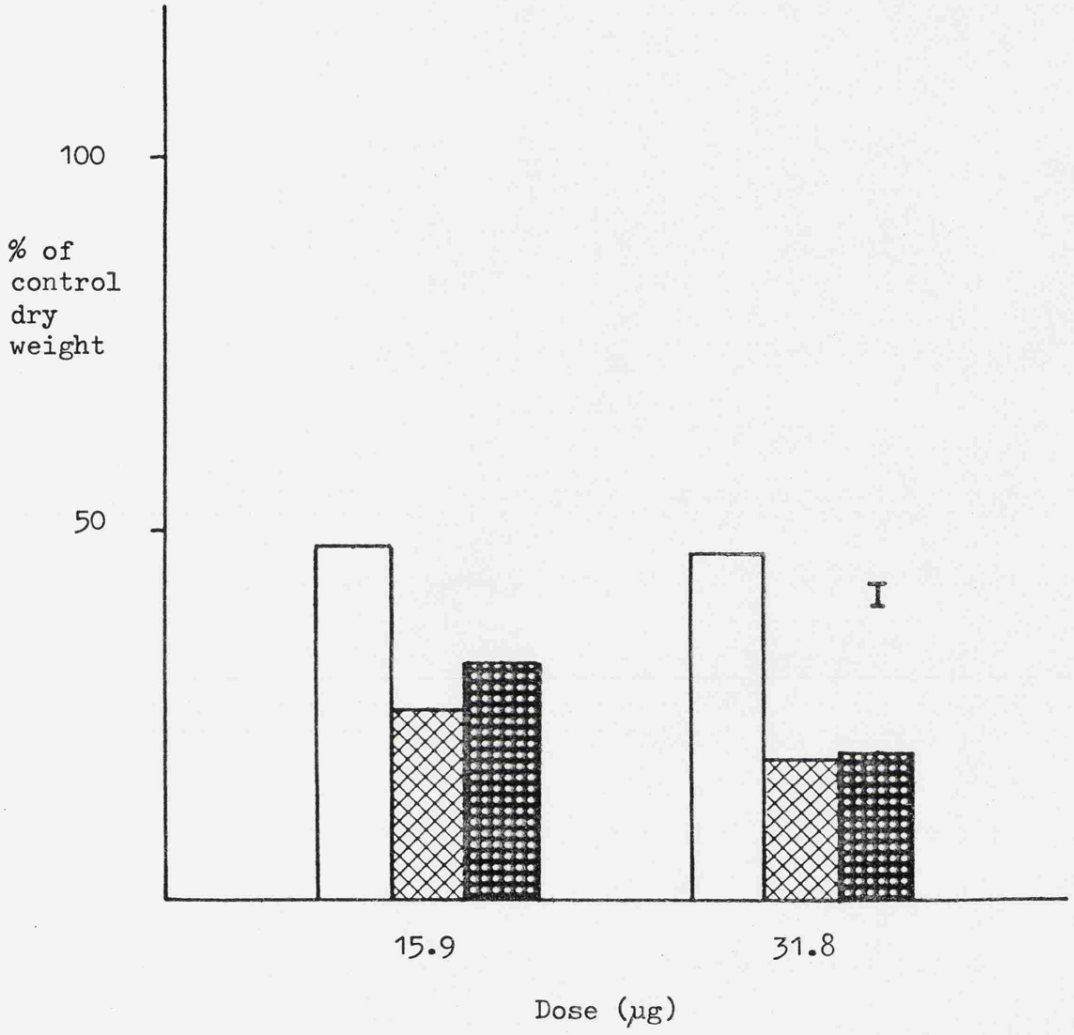


Figure 4.5: The response of radish (2 foliar leaves) to MCPA at two doses applied to three positions

Positions: cotyledons

foliar leaves

cotyledons and foliar leaves

(lower dose only) that the treatment of both cotyledons and foliar leaves is less effective than treatment of the foliar leaves only. Thus the cotyledons appear to be less effective sites for treatment with MCPA. In practice the foliar leaves, when expanded, are much larger than the cotyledons, so they are more important in terms of target area also. Reasons for the difference in effect could be due to differences in either penetration or movement of the MCPA. With both possibilities the ageing of the cotyledons, consequent changes in surface structure, and reduced importance as a source of food in the plant would be likely to contribute to the observed effect.

The second experiment showed that treatment to the midvein was more effective than treatment to the leaf lamina (Fig. 4.6). Again a number of factors could be involved. It is well established that the surfaces over veins have a reduced cover of surface wax deposits than leaf laminae (Martin and Juniper, 1970). In addition herbicide entering tissue associated with the vein may be more readily taken into the transport system, particularly the xylem system. Thirdly treatment to the midvein is closer to the shoot apex which is likely to be a major site of MCPA activity, and this may contribute to the result. However, considering the small target area of the midvein it is unlikely that the difference in effect observed would be of major importance in determining the overall response of an MCPA spray.

4.3.1.5 The effect of surfactant concentration. Surfactant concentration is a factor most likely to be affected by changes in volume rate, since most commercial herbicide formulations contain surfactants at levels dictated by the normal recommended spray volume. Thus in this experiment a typical concentration of Agral, that is 0.1% v/v, was compared with two higher concentrations of 1.0 and 10.0% v/v and also

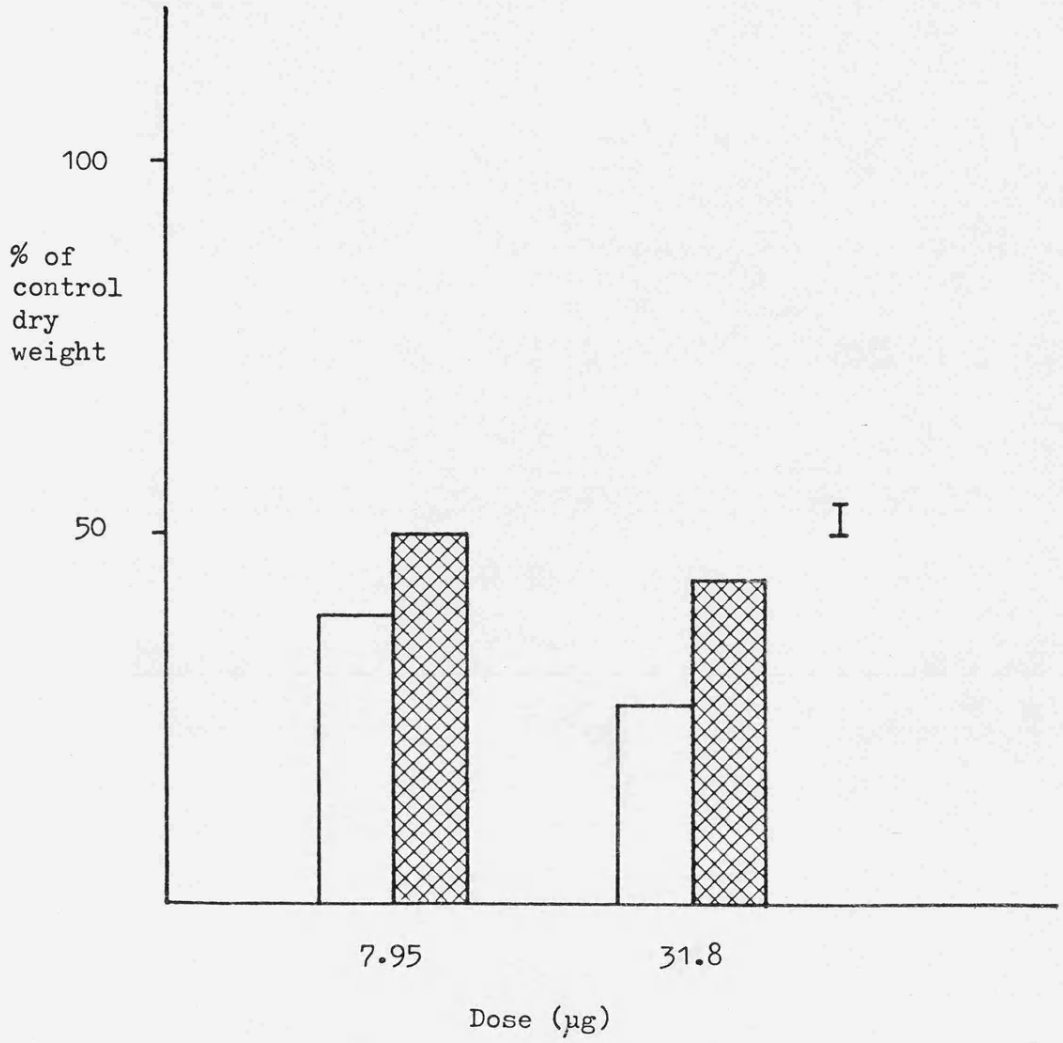


Figure 4.6: The response of radish (2 foliar leaves) to MCPA at two doses applied to two positions on the foliar leaves.

Positions: Mid vein

Lamina between veins

one lower concentration of 0.01% v/v. These four solutions contained 70 $g.l^{-1}$ MCPA and were applied in doses of 7.95, 15.9 and 31.8 µg to the first pair of foliar leaves of radishes. The radishes had two fully expanded foliar leaves and a mean fresh weight of 1.08 grams at the time of treatment; there were ten replicates. In this experiment only fresh weight was assessed (after 28 days) since in no previous experiment had dry weight results differed from fresh weight.

The results (Fig. 4.7) showed a highly significant dose response ($p < 0.001$) but no difference between surfactant concentration. This is a somewhat surprising result since the experiment studied a 1000-fold concentration range and surfactants are known to influence many factors including spreading, wetting, penetration and movement (Foy and Smith, 1969). The static surface tension of the solutions in this experiment were 32.5 mNm^{-1} for the 0.01, 0.1 and 1.0% concentrations and 32.3 mNm^{-1} for the 10.0% concentration, so it seems that all concentrations were above the cricital micelle concentration, and thus it may be that Agral concentration does not affect the biological performance of MCPA above its C.M.C.. There was some evidence of surfactant or surfactant-induced damage at the site of drop deposits after 4-5 days visible as indentation in the leaf surface, but clearly this did not affect MCPA activity.

4.3.2 Experiments with difenzoquat on wild oat

4.3.2.1 The dose response. A range of doses of difenzoquat (5.7-91.0 µg a.i.) was applied as 8-128 drops of 300 µm diameter to wild oat plants having $2\frac{1}{2}$ leaves. The treatment contained 50 $g.l^{-1}$ of difenzoquat cation and 0.5% v/v 'Agral'. The drops were applied either to leaf 2 in a confined area 30-60 mm from the leaf ligule or evenly spread over leaves 1 and 2. The experiment included untreated controls and eight replicates.

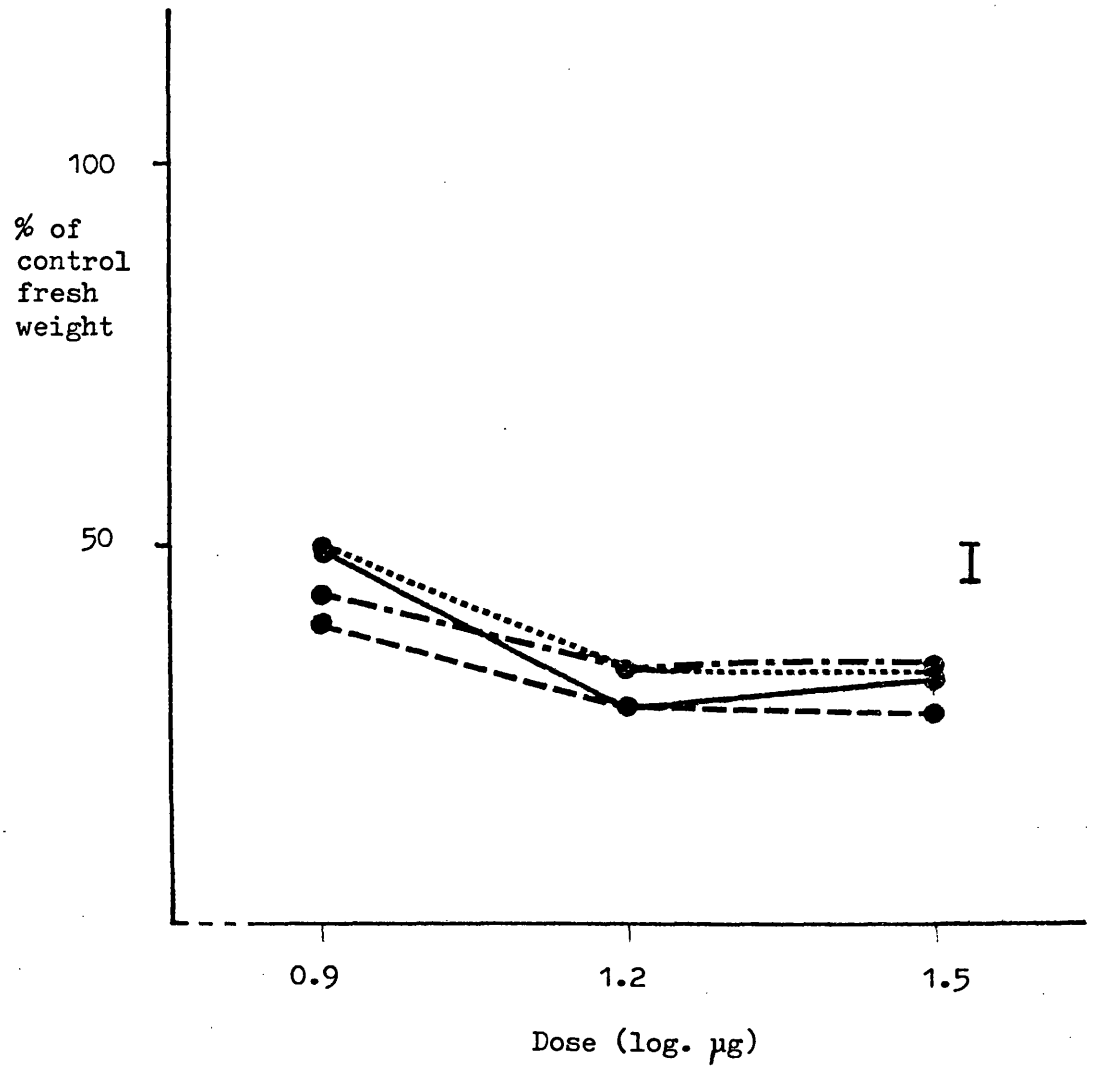


Figure 4.7: The response of radish (2 foliar leaves) to MCPA at three doses using four concentrations of 'Agral' surfactant.

Fresh weight was assessed 26 days after treatment. Analysis of variance of the fresh weight data showed a highly significant dose response ($p < 0.001$) with a maximum reduction in growth inhibition occurring at a dose of 45.5 - 91.0 µg (See Figure 4.8). There was no significant difference between the two positional variations although it appeared that the maximum growth inhibition response had been surpassed at the highest dose with the treatment to both leaves.

4.3.2.2 The effect of concentration. Difenzoquat was applied to wild oat plants at three doses (5.7, 11.4 and 22.7 µg per plant) in each of four concentrations (25, 50, 100 and 200 $g.l^{-1}$). The treatments were composed of various numbers of 300 µm drops applied to the third leaf of wild oat plants, the treated area being 20-40 mm from the ligule. The plants had 4 leaves and 1-3 small tillers, and each treatment was replicated ten times. Shoot fresh weight was assessed 28 days after treatments.

Analysis of variance showed a highly significant dose response ($p < 0.001$) and a significant effect of concentration ($p = 0.05$). As concentration of difenzoquat was increased the herbicide was less effective, causing less growth inhibition with the highest concentration than with the lowest (Figure 4.9).

The mechanism of this concentration effect is not immediately clear since the experiment involved several variables. Firstly, as concentration is increased, the number of drops for a given dose is decreased so that the total leaf area in contact with herbicide deposit is reduced. Secondly the amount of surfactant varied in relation to the amount of herbicide, since the surfactant concentration was maintained at 0.5% v/v in all solutions. Thirdly the evaporation rate may have been

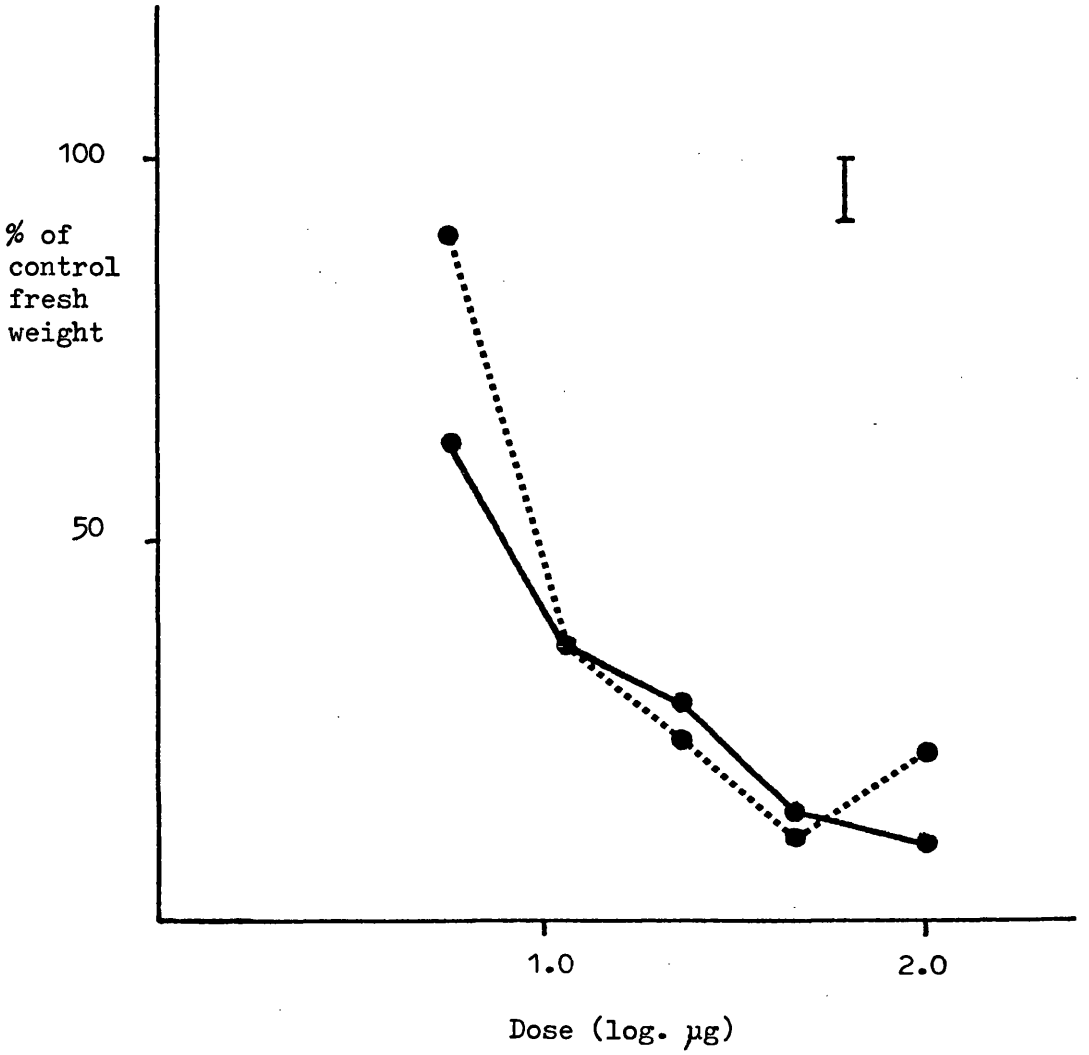


Figure 4.8: The response of wild oat ($2\frac{1}{2}$ leaves) to a range of doses of difenzoquat, applied as 300 µm drops using a concentration of 50 gl^{-1}.

Two positions were treated:

Leaf 2: 30-60mm from ligule ——

Leaf 1 and leaf 2 .....

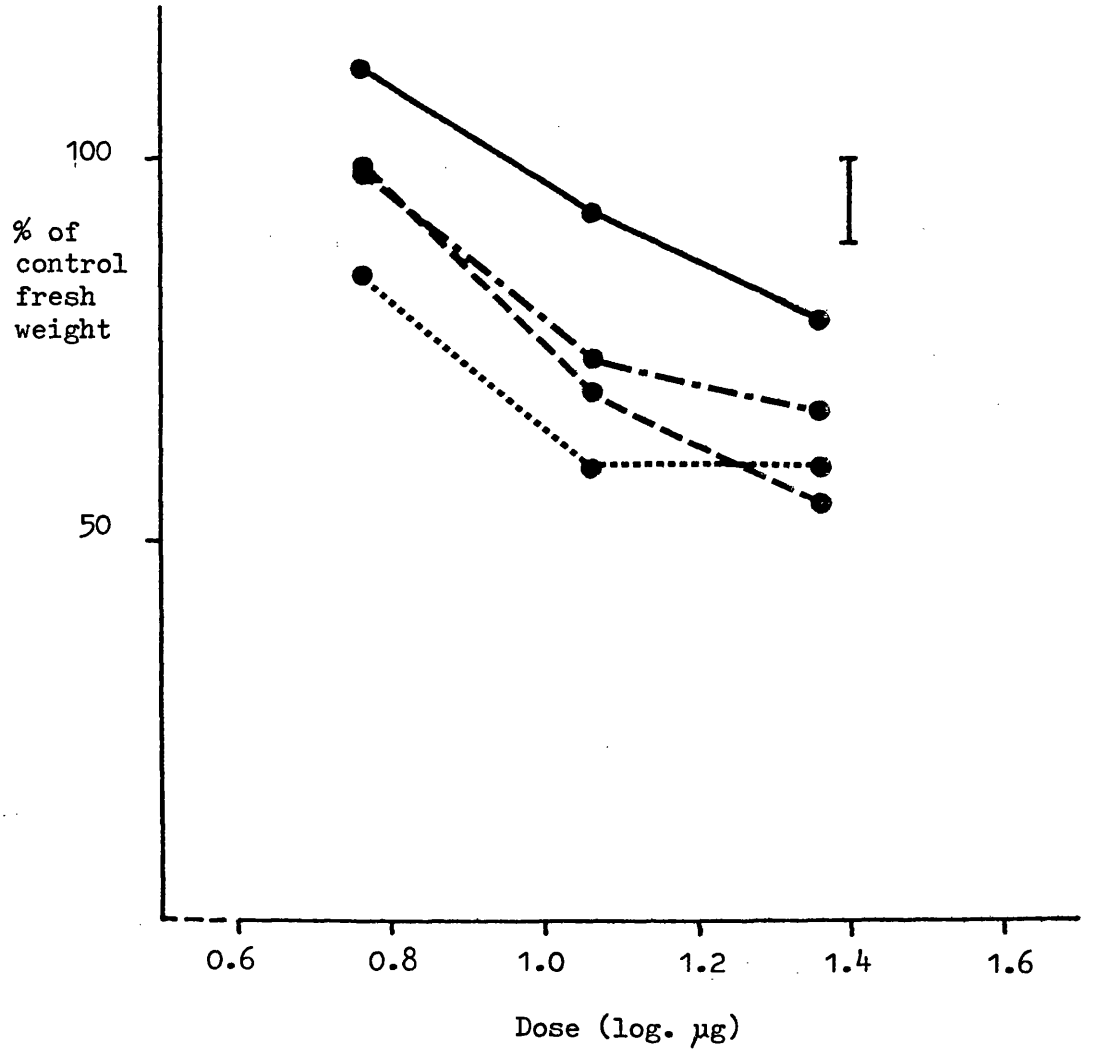


Figure 4.9: The response of wild oat (4 leaves) to difenzoquat at three doses and four concentrations, using 300 µm drops.

Concentrations: 25 gl^{-1} .....

50 gl^{-1} – – –

100 gl^{-1} .–.–

200 gl^{-1} ——

different between the various solutions and finally drop spread may have been altered by herbicide concentration differences.

The effect of surfactant concentration is studied in Section 4.3.2.5. Section 4.2 has already suggested that drop spread and evaporation rates are unlikely to be of major importance here. Variation in the area of contact of the spray deposit may affect performance either by influencing the rate of herbicide uptake or through physiological effects. One most important observation from this experiment was that the different concentrations caused varying amounts of necrosis at the site of treatment. Furthermore it was noticed that at 3-4 days after treatment the severity of necrosis around drops was more severe for the most concentrated solutions than for the more dilute solutions. It was considered likely that these effects were connected with the final growth inhibition and therefore a further experiment was carried out with the intention of quantifying these differences in necrosis.

This second experiment involved the application of the two extremes of the concentration range studied, namely 25 and 200 $g.l^{-1}$. Only a single dose was applied (22.7 µg) and drop size was maintained at 300 µm. Treatments were applied to leaves 1, 2 and 3 (30-50 mm from the ligule in each case) of wild oat plants with 3 leaves and 1 small tiller. Ten replicates were harvested after 7 days and the treated leaf removed. These leaves were then photographed as a visual record of degree of necrosis, and following this fresh and dry weight were determined for each treated leaf to assess the water content. A further nine replicates were grown on and harvested 30 days after treatment as a record of the effects of the treatments on growth inhibition.

The ratio of fresh weight : dry weight for the treated leaves

and untreated control leaves 7 days after treatment is shown in Figure 4.10. The data show that water content was reduced in treated leaves, more severely in the 25 $g.l^{-1}$ treatments than the 200 $g.l^{-1}$ ones. This is contrary to the expected result comparing the observed severity of necrosis caused by individual drops for the two concentrations. The photographic records of the treated leaves (Plate 4.1) reveal that individual drops tended to cause greater necrosis with the higher concentration of difenzoquat. However, since there are more drops of the more dilute solution the combined effect of these drops produced a greater reduction in water content than with the more concentrated solution.

With both concentrations of difenzoquat the reduction in water content was most severe on the oldest leaf (leaf 1) and least severe on the youngest leaf (leaf 3). This correlates with the observed degree of necrosis in Plate 4.1, as necrosis was most severe on leaf 1 and least severe on leaf 3.

The final assessment of shoot fresh weight 30 days after treatment with the second batch of treated plants (Figure 4.11) showed that for leaves 2 and 3 the lower concentration was more effective as was found in the preceding experiment. On leaf 1 difenzoquat was much less effective than on the younger leaves, and here a slightly greater effect on growth inhibition was observed with the higher concentration. These differences in performance of difenzoquat between leaves are discussed further in section 4.3.2.4 where other experiments on the effect of varying position of deposit are described.

4.3.2.3 The effect of drop size. Wild oat plants with $3\frac{1}{2}$ leaves and 2 small tillers were treated with three doses of difenzoquat (6.7, 13.4 and 26.8 µg) placed on the third leaf 10-40 mm from the ligule. Three

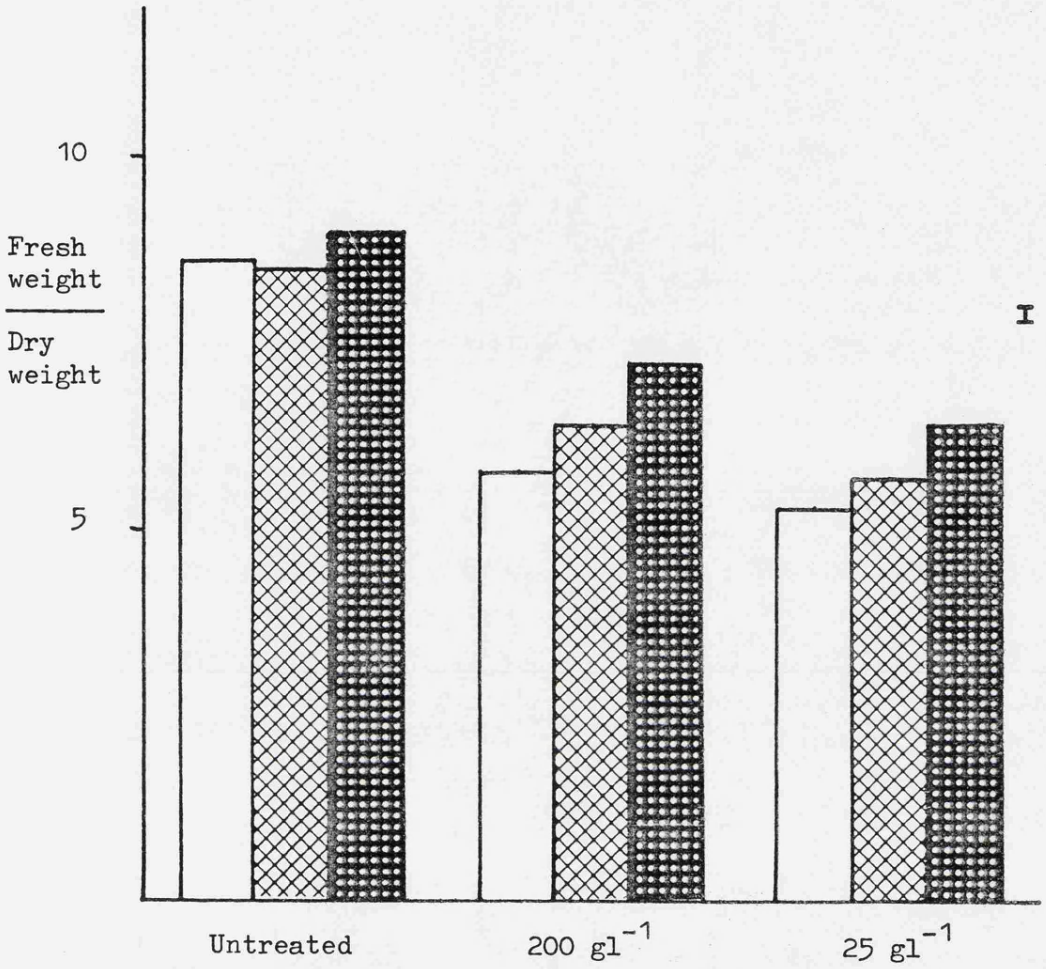


Figure 4.10: The effect of two concentrations of difenzoquat on the fresh weight/dry weight ratio of leaves of wild oat (3 leaves). The dose applied was 22.7 µg per plant.

Plate 4.1 Treated leaves from wild oat plants showing necrosis 7 days after application of difenzoquat at two concentrations.

a 25 $g.l^{-1}$ difenzoquat; leaf 1

b 25 $g.l^{-1}$ difenzoquat; leaf 2

c 25 $g.l^{-1}$ difenzoquat; leaf 3

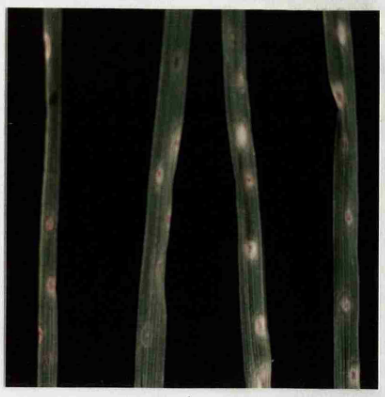
d 200 $g.l^{-1}$ difenzoquat; leaf 1

e 200 $g.l^{-1}$ difenzoquat; leaf 2

f 200 $g.l^{-1}$ difenzoquat; leaf 3

g untreated leaves (left to right : leaf 1, 2 and 3)

a



d

b

e

c

f

g

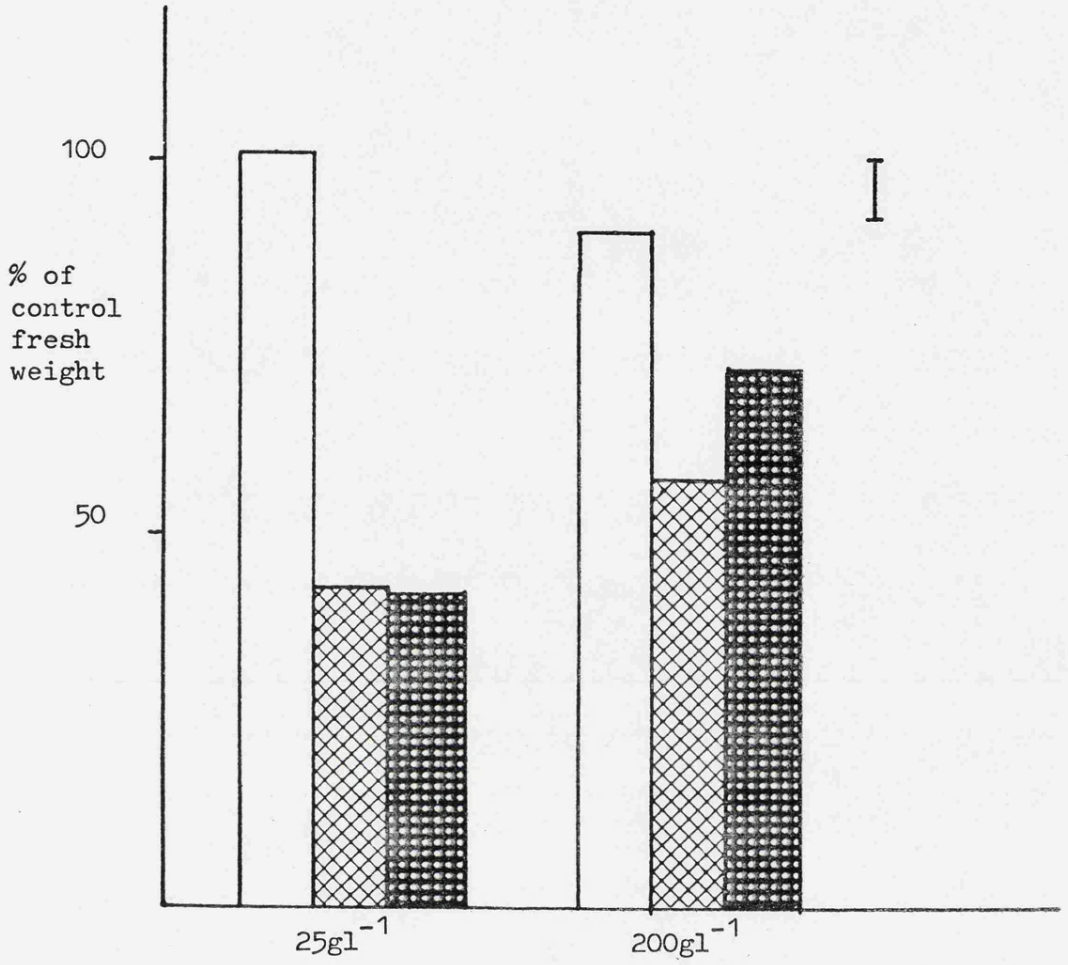


Figure 4.11: The response of wild oat (3 leaves) to difenzoquat at a dose of 22.7 µg per plant and two concentrations, applied to three positions.

Positions:
leaf 1
leaf 2
leaf 3

drop sizes were applied (200, 318 and 400 μm diameter) giving exact multiples of volume. The treatment solution contained 50 $g.l^{-1}$ of difenzoquat cation and 0.5% v/v of 'Agral'. Shoot fresh weight was assessed 28 days after treatment.

Analysis showed a highly significant dose response ($p<0.001$) and a significant effect due to drop size ($p = 0.01$). Although there was no significant interaction of dose and drop size the results (Figure 4.12) indicate that the greatest difference between drop sizes was at the lower doses, the smallest drop size (200 μm) being the most effective and the largest drop size (400 μm) the least effective.

It is likely that this result is connected with the degree of dispersal of the herbicide deposit on the leaf surface, as suggested for the effect of herbicide concentration in the preceding section. There are fewer drops with the larger drop size and, as shown in Section 4.2, the concentration of the dried deposit tends to increase with drop size. In addition it was found that necrosis a few days after treatment was more severe with the larger drops, again similar to the effect of herbicide concentration.

4.3.2.4 The effect of position of deposit. Four experiments are described in this Section, looking at various aspects of the influence of the site of treatment of wild oat plants. Two of these experiments compared treatments to leaf 1, leaf 2 or leaf 3 of plants with $3\frac{1}{2}$ leaves (first experiment) and $2\frac{1}{2}$ leaves (second experiment). Additional treatments included were to all three leaves (drops being evenly dispersed) in the first experiment and to the outer surface of the sheath of leaves 1 and 2 in the second experiment. Doses were 11, 21 and 43 μg in experiment 1 and 2.8, 5.7, 11.4 and 22.7 μg in experiment 2. The third experiment compared distance from the leaf ligule on leaf 2 of $3\frac{1}{2}$-leaf

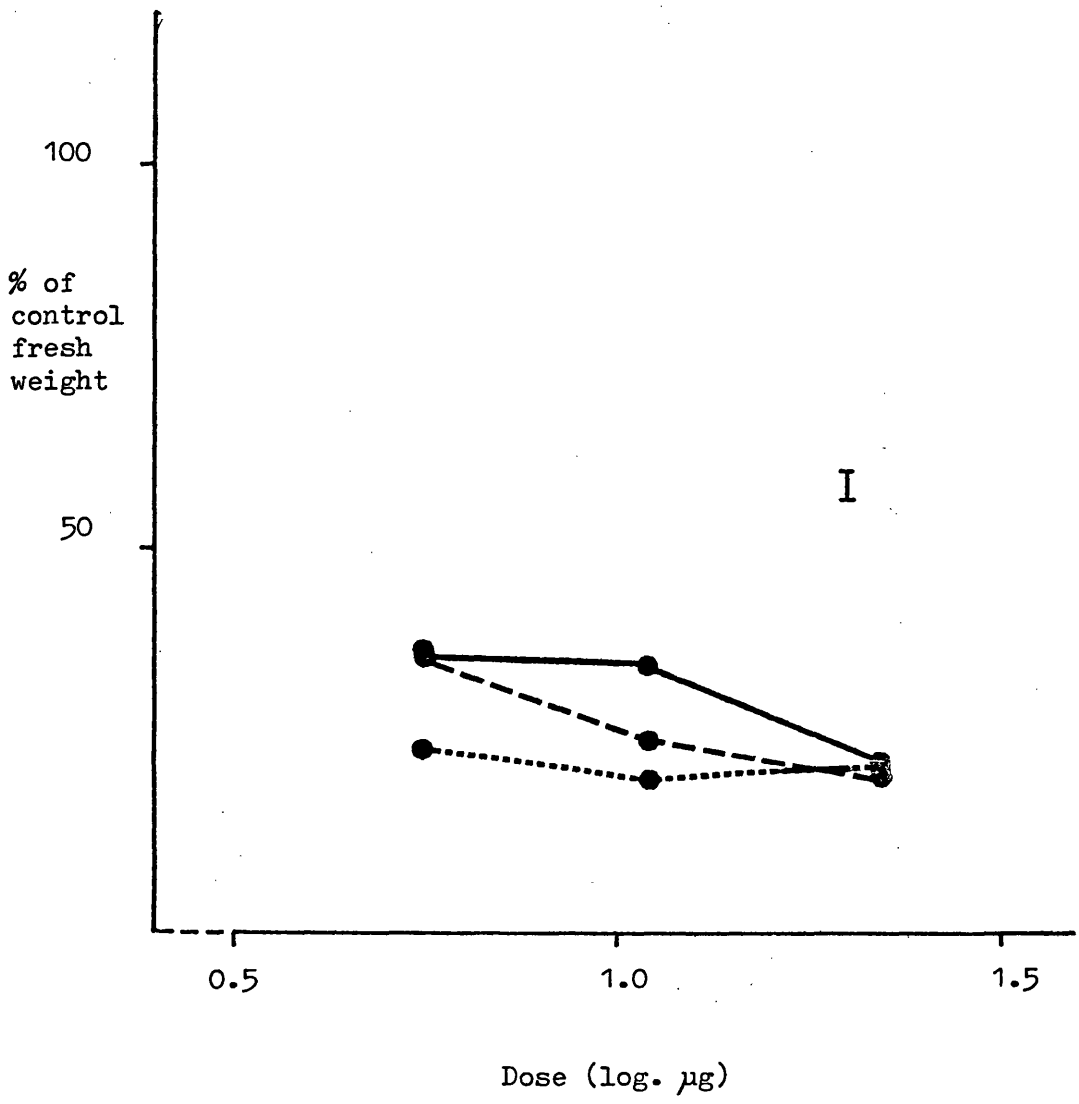


Figure 4.12: The response of wild oat ($3\frac{1}{2}$ leaves) to difenzoquat at three doses and three drop sizes using a concentration of $50gl^{-1}$.

Drop sizes: 200 µm ·····

318 µm – – –

400 µm ——

plants, treatments being applied 0-30 mm, 30-60 mm or 60-90 mm from the ligule. In the fourth experiment 2-leaf plants were treated on leaf 2 over areas 30-90 mm or 50-70 mm from the ligule to compare different degrees of dispersion of drops over the leaf surface. In all experiments the drop size was 300 μm and solutions containing 50 $g.l^{-1}$ difenzoquat were used in experiments 1, 2 and 3 whilst two solutions of 25 and 200 $g.l^{-1}$ were used in experiment 4. There were 8 replicates in each of the first two experiments and ten in the second two. The experiments were assessed for shoot fresh weight after 20, 22, 20 and 22 days for experiments 1-4 respectively.

The results of the first two experiments showed that the older leaves are less effective sites for difenzoquat activity. This is most pronounced with leaves 1 and 2 in the first experiment (Figure 4.13) and leaf 1 in the second experiment (Figure 4.14). The greater activity of leaf 2 in the second experiment is probably due to the younger plant stage treated. It appears that spreading the drops over all three leaves (Figure 4.13) was no more effective than treatment to the most effective leaf alone, that is leaf 3. However it is interesting that at the middle dose (21 μg) with the treatment of all three leaves, leaf 3 received only 7 μg of difenzoquat, yet the effect of this treatment was much greater than that of the lowest dose (11 μg) to leaf 3 alone. Thus the herbicide applied to leaves 1 and 2 (7 μg each) must have contributed a substantial part of the total effect with this split treatment, probably greater than when these older leaves were individually treated with a much higher dose. This probably indicates that a given site has the capacity to export a certain amount of difenzoquat, which may be reduced if the dose at that site is increased. Further evidence for this comes from the observed results that the maximum inhibition of growth occurred below the highest dose with leaf 2 in Figure 4.13 and

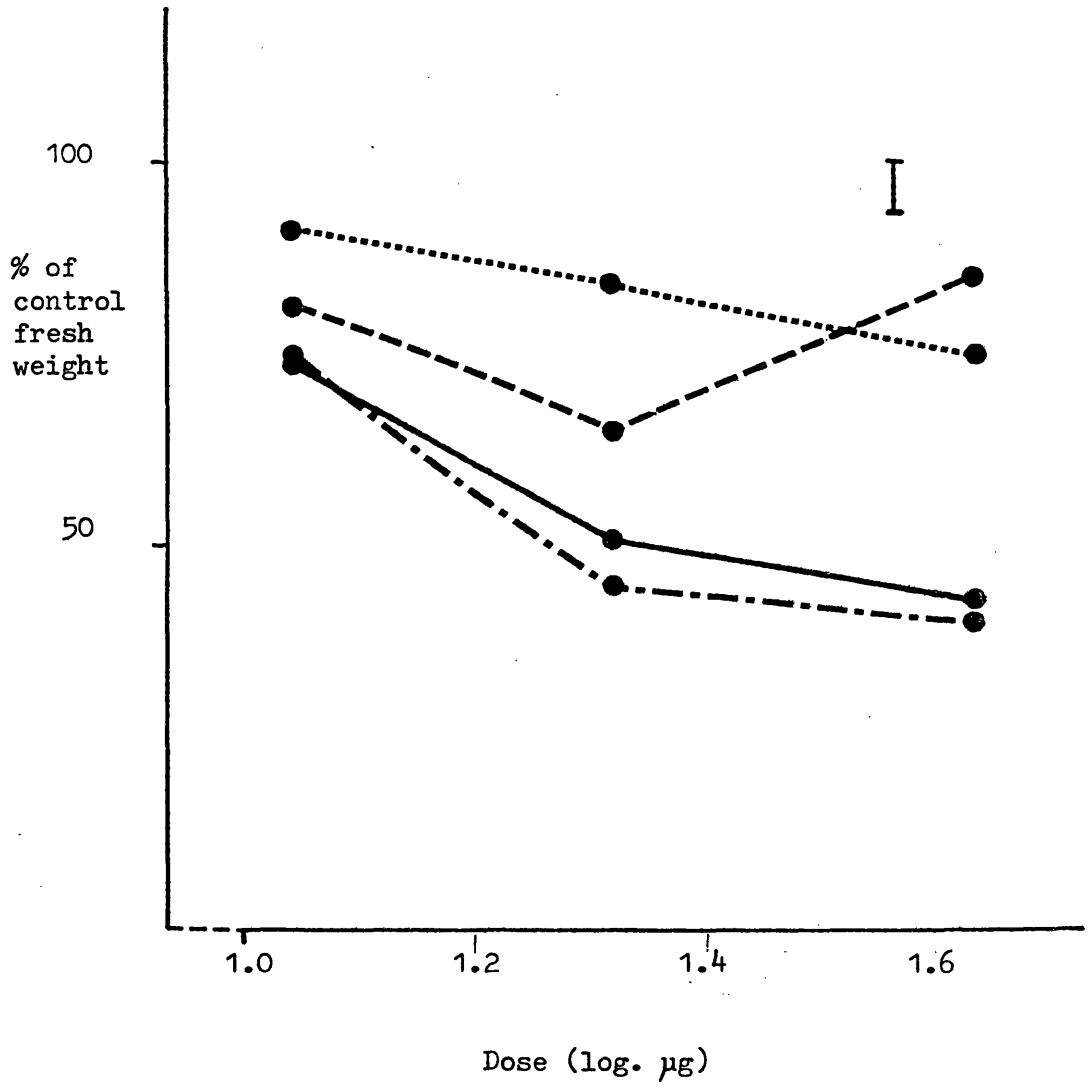


Figure 4.13: The response of wild oat ($3\frac{1}{2}$ leaves) to difenzoquat at three doses using a concentration of $50gl^{-1}$ and 300 µm drops, applied to four positions.

Positions:
- Leaf 1 .....
- Leaf 2 – – –
- Leaf 3 – · –
- All three leaves ———

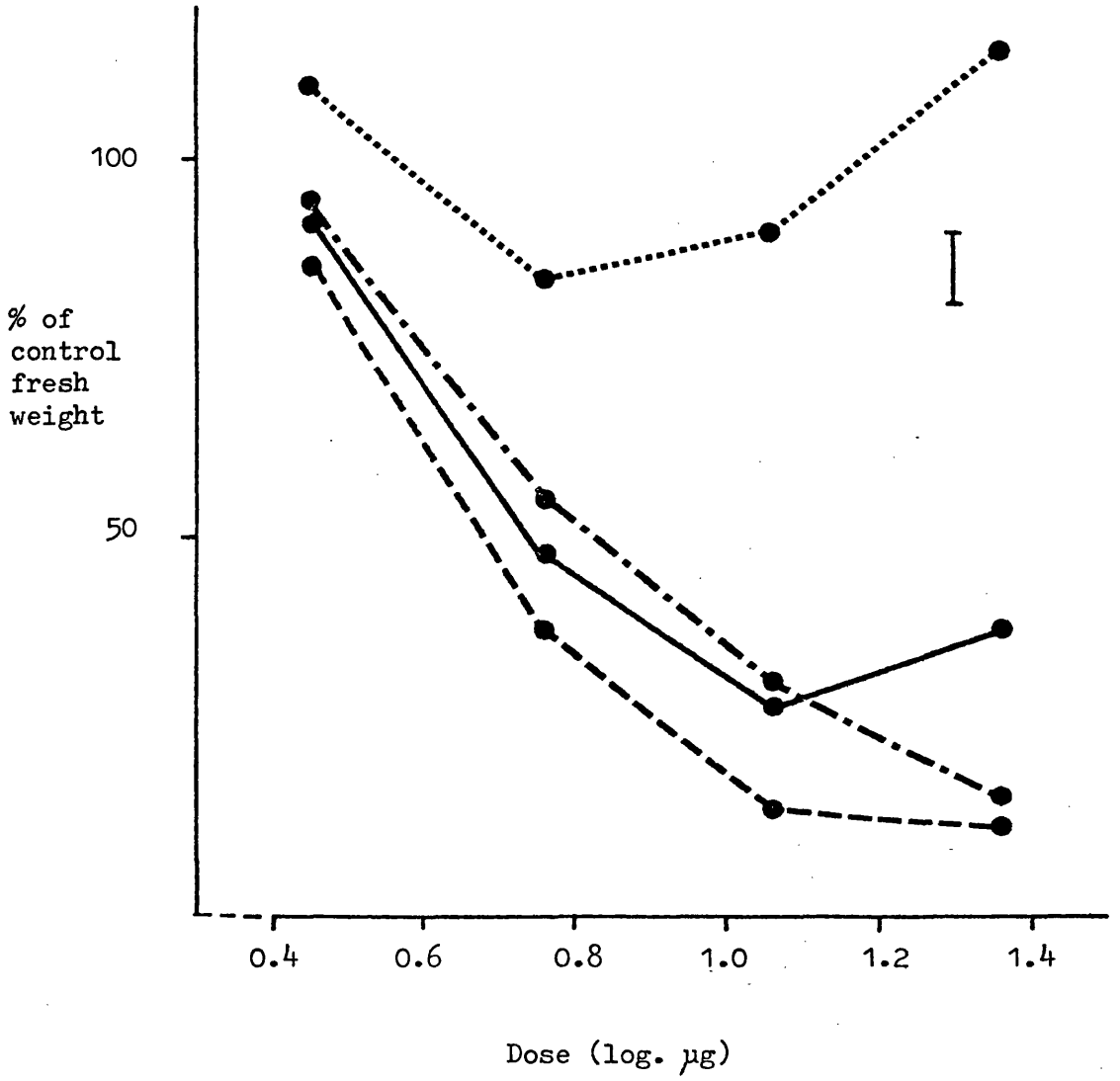


Figure 4.14: The response of wild oat ($2\frac{1}{2}$ leaves) to difenzoquat at four doses using a concentration of $50gl^{-1}$ and 300 µm drops, applied to four positions.

Positions:
- Leaf 1 .....
- Leaf 2 – – –
- Leaf 3 —·—
- Leaf sheath ——

leaf 1 in figure 4.14. Since in Section 4.3.2.2 it was shown that necrosis at the site of treatment was generally more severe on older leaves, it would seem probable that this feature of difenzoquat application in high concentrations might also explain the results with site of application.

The treatment to the leaf sheath of leaves 1 and 2 in the second experiment (Figure 4.14) was not significantly different from the treatments to leaves 2 and 3, a surprising result considering the proximity of this site to the shoot apical meristem, the presumed site of action. However, the increased proximity of the drops in this more restricted treatment may have accounted for this result.

Experiment 3 (Figure 4.15) showed that difenzoquat was more effective when applied nearer to the base of the leaf lamina. This is perhaps to be expected since the site of action is the apical meristem, which is near to the basal end of the leaves. However, the mechanism of translocation of this herbicide is not yet fully known, and the reason for this result may not therefore be simply one of proximity. It has been suggested that differences in the surface wax may be responsible for such differences in herbicide performance along the leaf laminae of wild oat (Walter and Bischof, 1976). The least effective position in this experiment (60-90 mm from the ligule) showed a negative dose response, probably due to restricted movement of herbicide from beneath each drop. Since difenzoquat moves largely in an apoplastic manner (Sharma et al, 1976) redistribution within the leaf would be greater towards the leaf base where the transpiration flow rate is greater.

The results of the fourth experiment (Figure 4.16) are difficult to interpret since both the degree of dispersion of drops and proximity

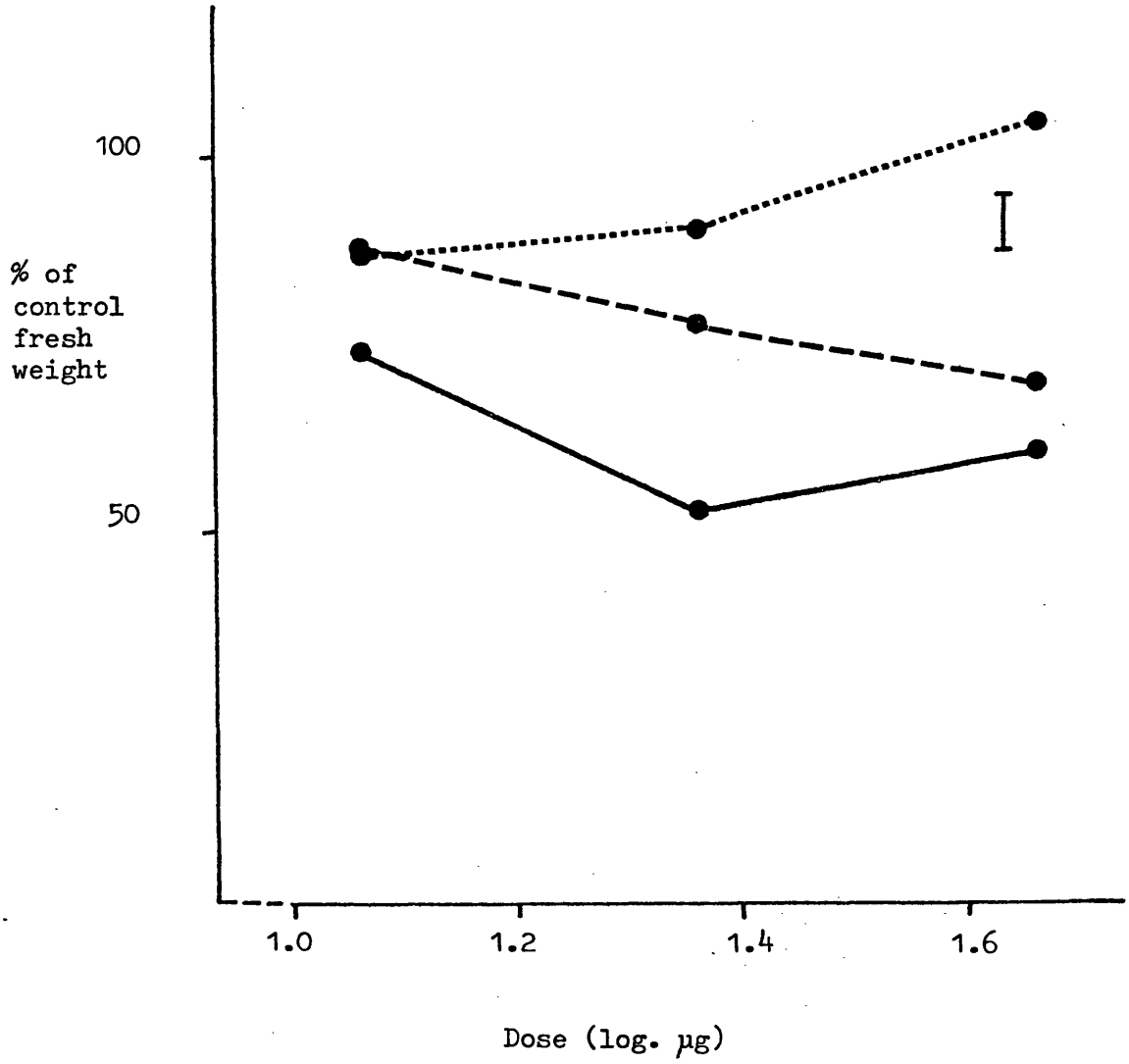


Figure 4.15: The response of wild oat ($3\frac{1}{2}$ leaves) to difenzoquat at three doses using a concentration of $50gl^{-1}$ and 300 µm drops, applied to three positions on leaf two.

Positions:
0-30 mm from ligule ——
30-60 mm from ligule – – –
60-90 mm from ligule .....

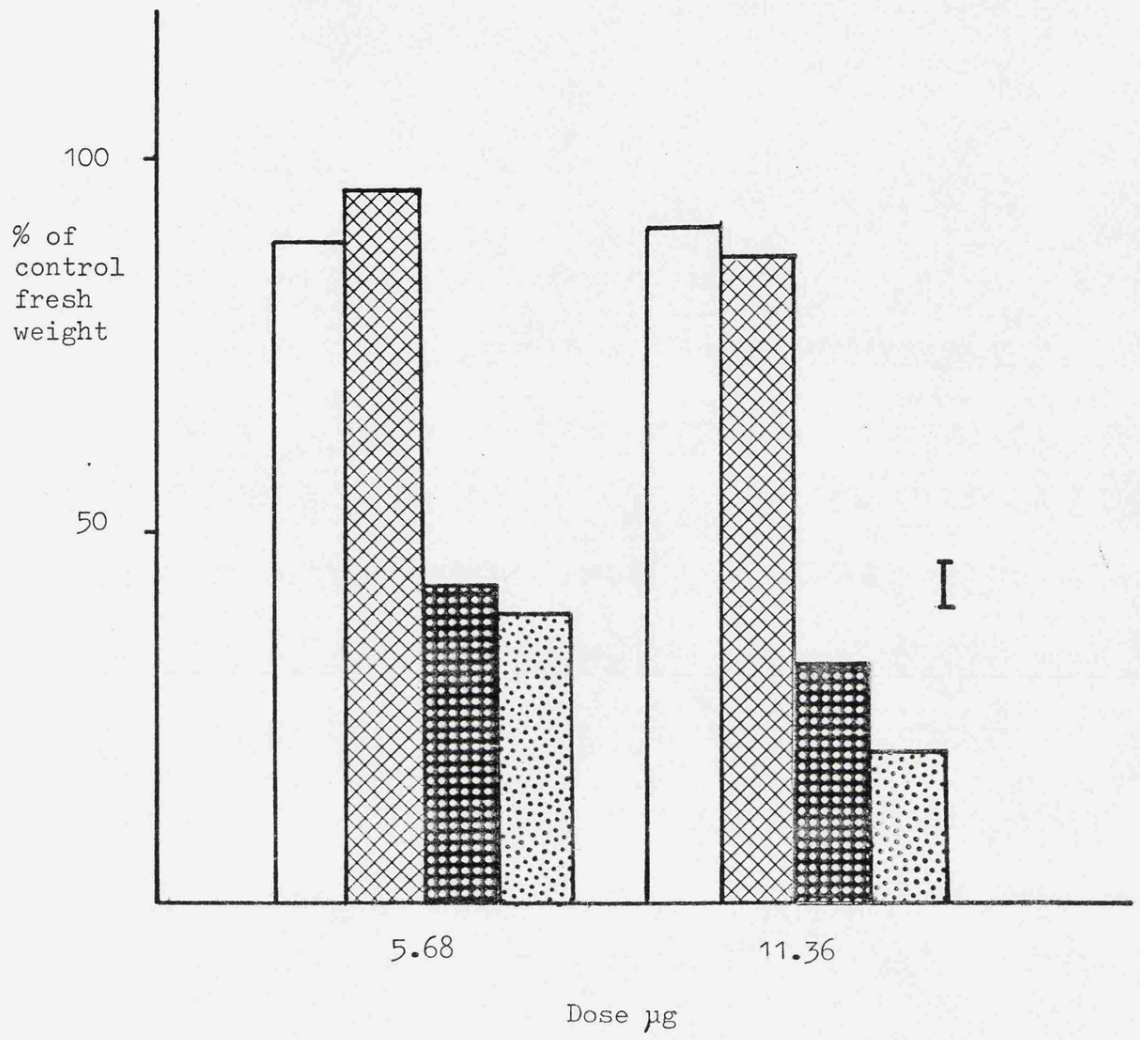
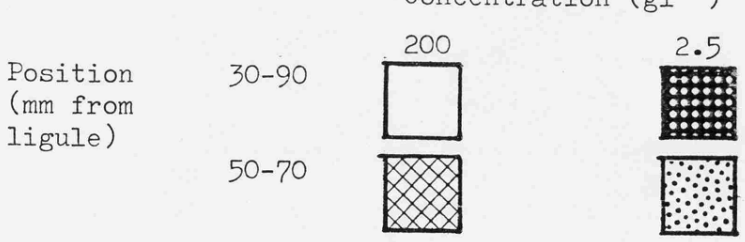


Figure 4.16: The response of wild oat (2 leaves) to difenzoquat at two doses using two concentrations applied to two positions on leaf two.



to the leaf base were altered, although the centre of the treated area remained a set distance from the ligule. However, there was a very large difference observed between the two concentrations of difenzoquat, as before the more concentrated treatment being the least effective. Despite the problems of interpretation there were no significant differences between the two dispersion treatments, this being unexpected from previous findings since with the more dispersed treatment not only were the drops further apart but also a proportion of the drops were close to the leaf base, previously found to be more effective. This result suggests that it is the concentration of the deposit within each drop that is important in determining performance rather than the degree of dispersion of the drops.

4.3.2.5 The effect of surfactant concentration. A number of experiments have already shown that difenzoquat is less effective when applied as fewer more concentrated drops. In all these experiments the surfactant concentration was 0.5% v/v for all solutions, this being the concentration normally used in spray solutions with this herbicide. If surfactants acted only through their effects on physical properties of the spray solution this would be a reasonable procedure. However it is widely held that surfactants affect many processes, including herbicide penetration into, and dispersion within plant tissues. It is possible that such effects might depend on the proportion of surfactant molecules to molecules or ions of active ingredient. To test this possibility two concentrations of difenzoquat previously found to differ markedly in activity, namely 25 and 200 $g.l^{-1}$, were applied to wild oat plants having $2\frac{1}{2}$ leaves. Agral was included in the 25 $g.l^{-1}$ solution at the normal 0.5% concentration, but at two concentrations in the 200 $g.l^{-1}$ solution, namely 0.5 and 4.0 v/v. Thus the 4.0% concentration maintained the same

mass ratio of surfactant to active ingredient as the 0.5% concentration with the 25 $g.l^{-1}$ difenzoquat solution. The treatments were applied to the second leaf as doses of 11.4 and 22.7 µg using 300 µm drops. Shoot fresh weight was assessed 18 days after treatment.

There was a significant effect due to difenzoquat dose (Figure 4.17). However, the higher surfactant concentration did not restore the loss in effect which occurred with the higher concentration as in previous experiments. Instead the effect was poorer with the higher Agral concentration. It appeared that the higher Agral concentration only served to enhance the local damage which was apparently responsible for the poorer effectiveness of the higher difenzoquat concentration, probably due to increased penetration of difenzoquat at this site, although some damage directly due to the surfactant cannot be ruled out.

A second experiment was designed to study the effect of a range of Agral concentrations on difenzoquat activity using a solution containing 50 $g.l^{-1}$ of the herbicide. Three doses of 11.4, 22.7 and 45.4 µg were applied as 300 µm drops to the third leaf of wild oat plants with 3 leaves, the drops being placed 40-80 mm from the ligule. Three concentrations of Agral were used (0.05, 0.5 and 5.0% v/v) and there were 8 replicates. The degree of necrosis around the treated areas was studied after 7 days and shoot fresh weight assessed after 18 days.

Figure 4.18 shows the results of the shoot fresh weight assessment, indicating a significant dose response and a marked reduction in activity of the lowest Agral concentration (0.05%) compared with the higher concentrations. This result is entirely without influence from any retention effect, and no detectable effect on drop spreading was apparent, indeed the surface tensions of the solutions were measured

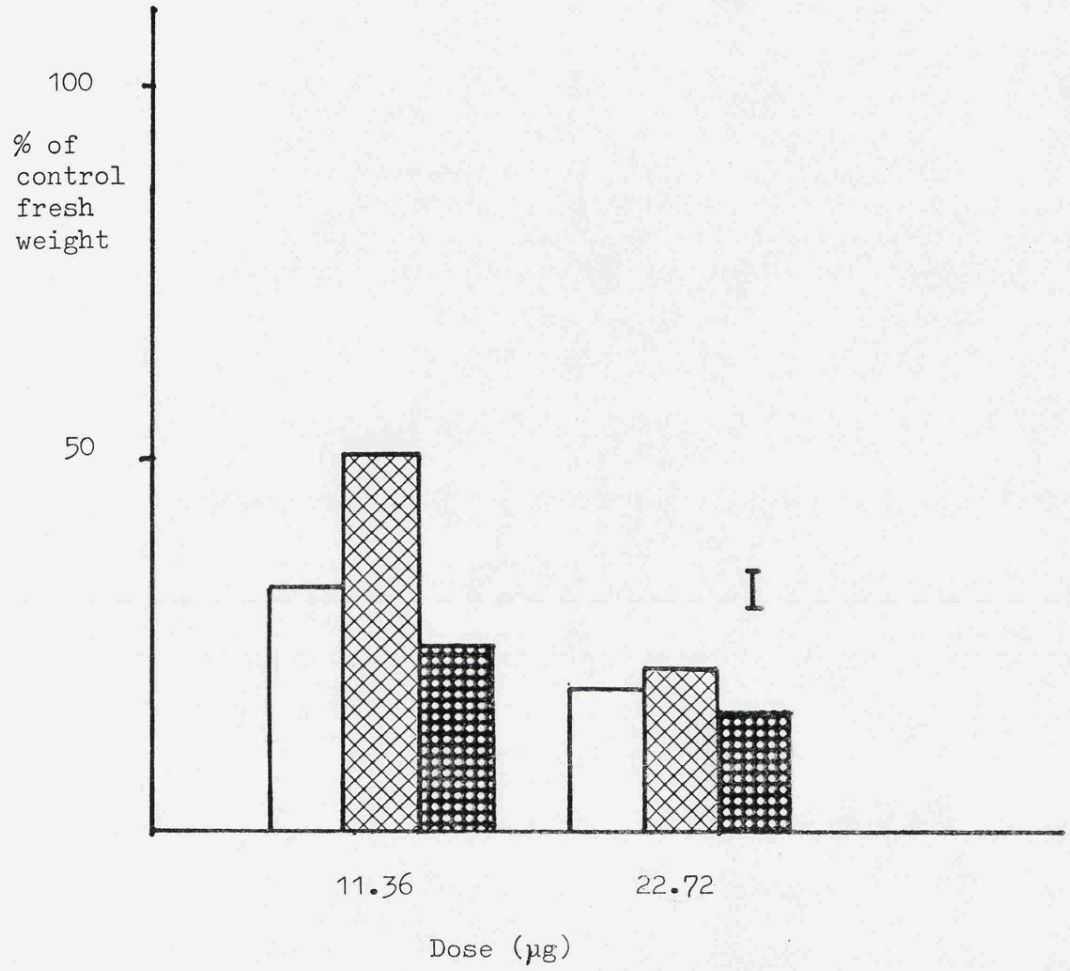


Figure 4.17: The response of wild oat ($2\frac{1}{2}$ leaves) to difenzoquat at two doses using two concentrations of both herbicide and 'Agral' surfactant

Solutions: $200gl^{-1}$ difenzoquat; 0.5% 'Agral'

$200gl^{-1}$ difenzoquat; 4.0% 'Agral'

$25\,gl^{-1}$ difenzoquat; 0.5% 'Agral'

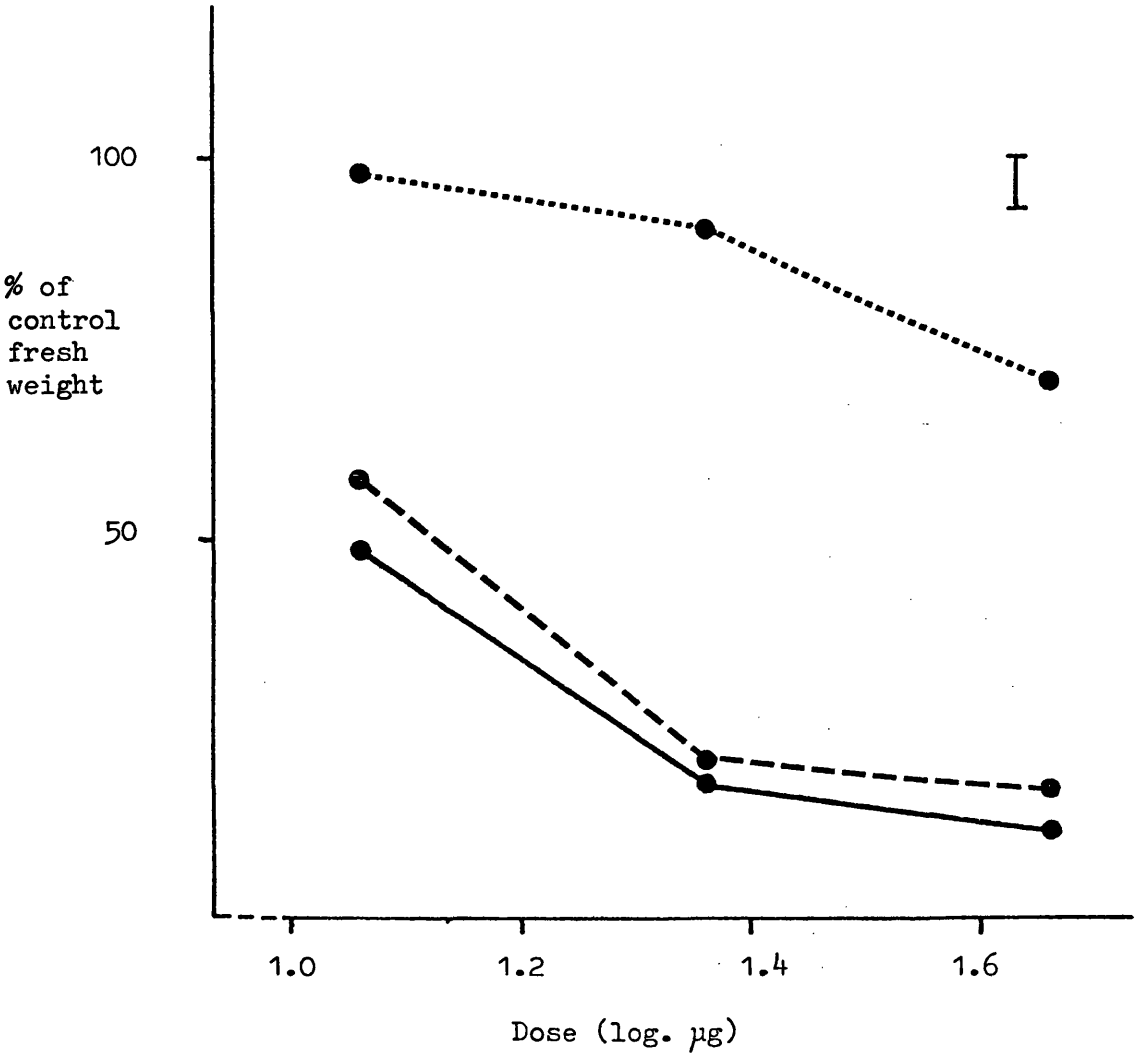


Figure 4.18: The response of wild oat (3 leaves) to difenzoquat at three doses using a concentration of $50gl^{-1}$ and three concentrations of 'Agral' surfactant.

'Agral' concentrations: 0.05% v/v .....

0.5 % v/v – – –

5.0 % v/v ———

as 34 mNm^{-1} for the 0.05% concentration and 35 mNm^{-1} for the other two solutions.

Observations on the degree of necrosis on the treated leaves revealed that this increased with increasing surfactant concentration.

To establish whether this increase in necrosis was due to the surfactant concentration alone or to some effect on the activity of difenzoquat a third experiment was conducted to compare the same three concentrations of Agral with or without difenzoquat. A single dose of 44 µg was applied as 64 drops of 300 µm diameter using a solution of 50 $g.l^{-1}$ difenzoquat. The drops were applied to the second leaf of plants with $2\frac{1}{2}$ leaves, using 0.05, 0.5 and 5.0% Agral concentrations. The degree of necrosis of the treated leaves was observed and photographed after 7 days and fresh weight assessed after 24 days.

The results again showed that the higher two concentrations of Agral were much more effective than the lowest concentration when applied with difenzoquat (Figure 4.19), indeed the lowest concentration did not cause a significant reduction in fresh weight compared with the treatments of surfactant alone. In contrast to the findings of the previous experiment it was in this case found that the maximum Agral concentration (5.0%) reduced the effect of difenzoquat compared to that with 0.5% Agral. This may be due to the application being made to the second leaf in this experiment rather than the third leaf as was the case in the previous experiment, because previous experiments showed that the second leaf is more prone to localised damage.

This experiment confirmed that necrosis at the site of treatment was mainly due to the combination of difenzoquat with Agral rather than the Agral alone, as seen in Plate 4.2. Although there is necrosis

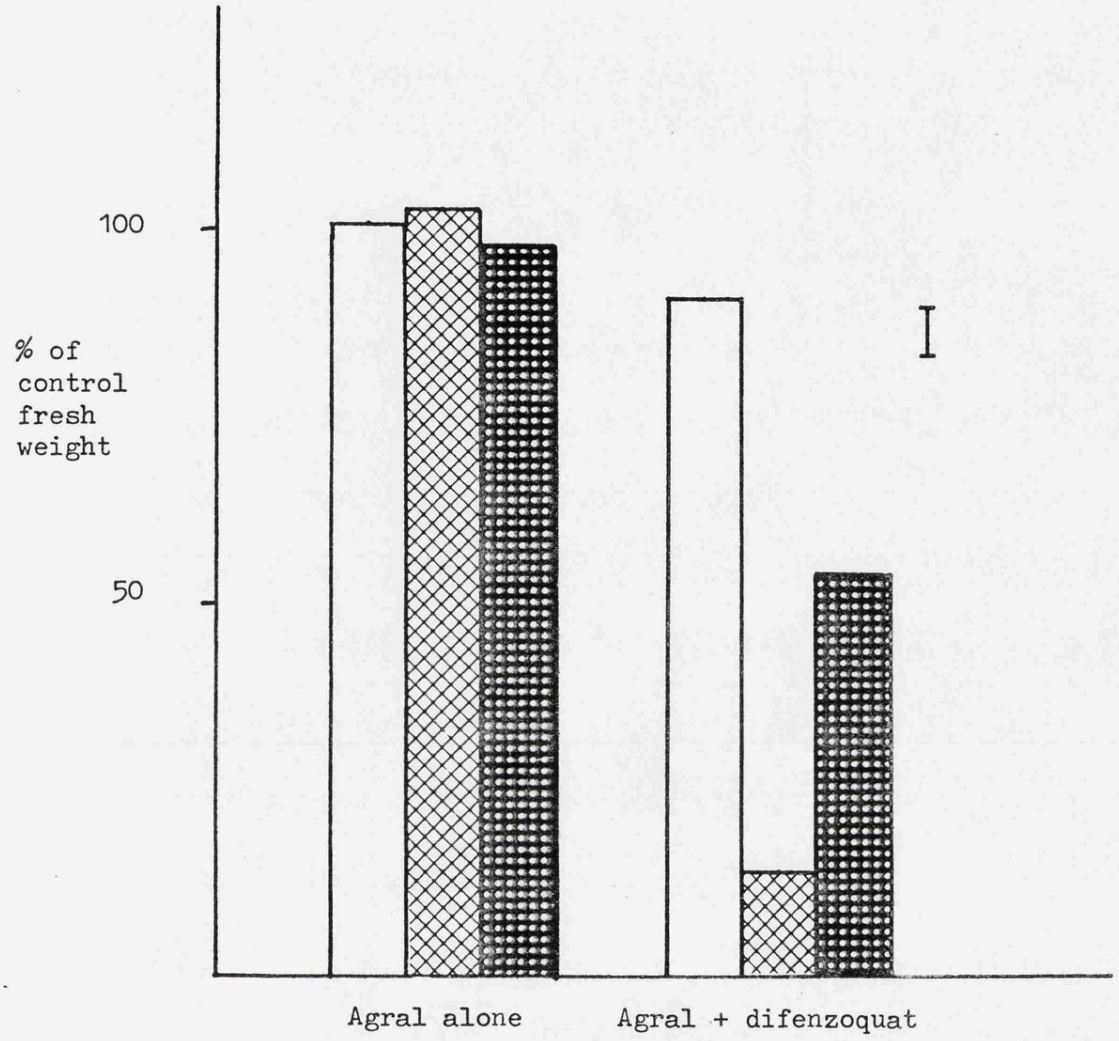


Figure 4.19: The response of wild oat ($2\frac{1}{2}$ leaves) to three 'Agral' concentrations with and without difenzoquat at a dose of 44 µg per plant.

'Agral' concentrations:
0.05% v/v
0.5 % v/v
5.0 % v/v

Plate 4.2 Treated leaves from wild oat plants showing necrosis 7 days after application of drops of Agral solutions at three concentrations with and without addition of difenzoquat at 50 $g.l^{-1}$.

a 0.05 % Agral

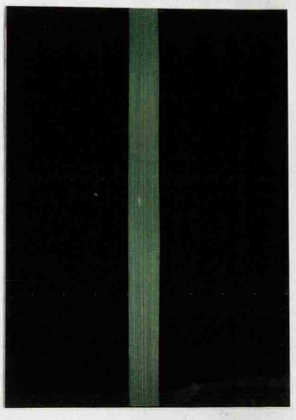
b 0.5 % Agral

c 5.0 % Agral

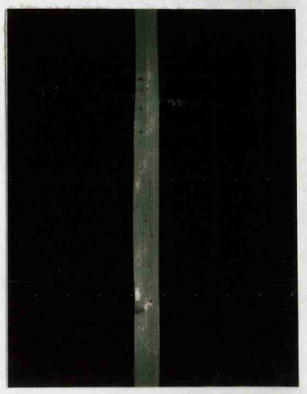
d 0.05 % Agral + difenzoquat

e 0.5 % Agral + difenzoquat

f 5.0 % Agral + difenzoquat.



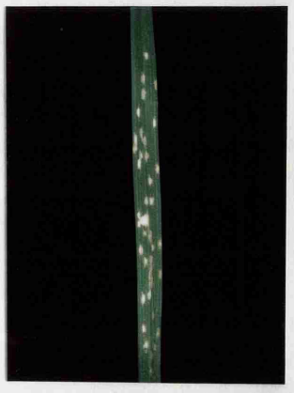
a



d

b

e



c

f

with Agral alone, particularly at the highest concentration, this is always restricted to the area of contact of the original drop, whilst in the presence of difenzoquat necrosis is much more severe, and spreads beyond the immediate area of contact.

4.3.3 Experiments with paraquat on radish

4.3.3.1 The dose response. A range of doses of paraquat (1.6 - 25.5 µg a.i.) was applied to radishes with two fully expanded foliar leaves having a mean dry weight per plant of 0.14 g. These doses were applied as 4-64 drops of 300 µm diameter of a solution containing 28 $g.l^{-1}$ paraquat and 0.1% v/v Agral, the drops being evenly dispersed over the first pair of foliar leaves. The experiment comprised ten replicates and included untreated control plants. Fresh and dry weights of foliage were determined 12 days after treatment.

The results (Figure 4.20) showed a significant dose response with both fresh and dry weight, as well as with the ratio of fresh weight to dry weight. This ratio is often used as a method of assessment of the effect of herbicides which have a desiccant action (as does paraquat) since it is a relative measure of plant water content. However in this experiment fresh weight : dry weight appeared to be less useful as an assessment criterion than fresh weight or dry weight alone, except at the higher doses. This is because of the narrow dose range over which the response changed from little to almost complete effect. With all three criteria the maximum response appeared to occur at around 6.4 µg per plant. With this dose three plants died out of the ten replicates. Below 6.4 µg all plants survived whilst at 12.7 and 25.5 µg six and ten plants died respectively. It was concluded that the dose range for subsequent experiments should be around or just below 6.4 µg per plant.

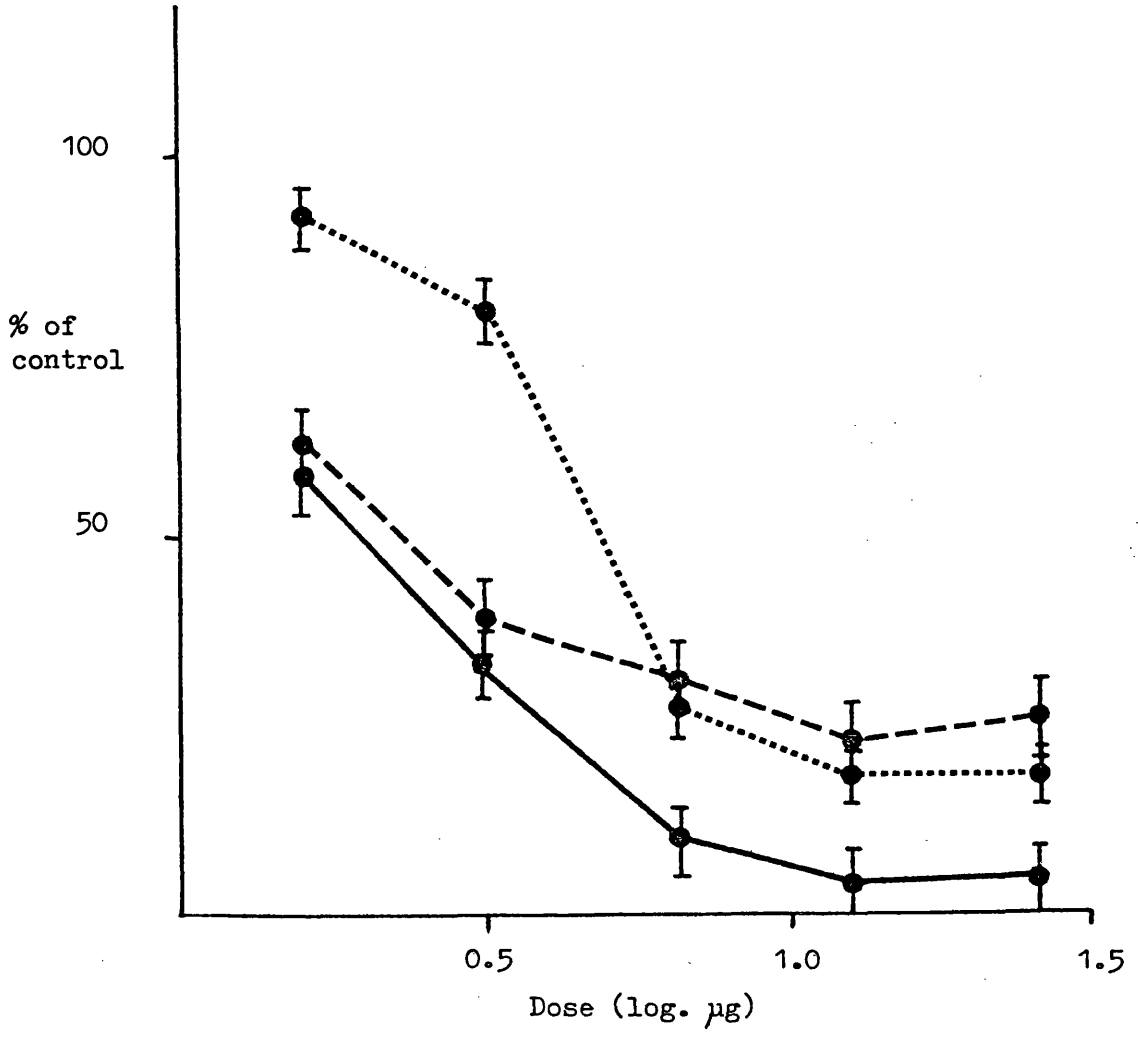


Figure 4.20: The response of radish (2 foliar leaves) to paraquat at a range of doses using three criteria of assessment.

Assessment criteria: Fresh weight ——
Dry weight – – –
Fresh weight/ dry weight .....

4.3.3.2 The effect of paraquat concentration. Two experiments were conducted here. In the first experiment two concentrations of 14 and 112 $g.l^{-1}$ paraquat cation were applied as three doses of 3.2, 6.4 and 12.7 μg per plant to radishes with 3-4 foliar leaves, the treatments being applied to the first pair of foliar leaves. The drop size used was 300 μm and there were 16 replicates. Fresh weight and dry weight were assessed after 15 days.

The results showed a highly significant dose response for fresh weight, dry weight and fresh weight : dry weight ($p < 0.001$ in each case) but whilst the effect of paraquat concentration was significant for all three criteria that for fresh weight was of highest significance ($p < 0.001$), followed by dry weight ($p = 0.01$) and then by fresh weight : dry weight ($p = 0.05$). Thus fresh weight again gave the most effective assessment. It must be stressed that plants were watered about 1 hour before assessment so as to ensure full plant turgor, and this would appear to be essential for this type of experiment with fresh weight assessment.

The results of the fresh weight assessment are shown in Figure 4.21, and indicate that the more concentrated solution (112 $g.l^{-1}$) was more effective than the more dilute solution (14 $g.l^{-1}$). Chlorotic symptoms appeared on the young, untreated plant parts after about 3 days and eventually these parts became necrotic on the most severely affected plants. It was apparent both from these observations and from the fresh weight assessment that paraquat movement to the younger parts was greater with the more concentrated solution.

A second experiment compared intermediate concentrations of 56 and 28 $g.l^{-1}$ with the lower concentration of the first experiment, 14 $g.l^{-1}$. The radishes in this experiment had 2-2$\frac{1}{2}$ foliar leaves and

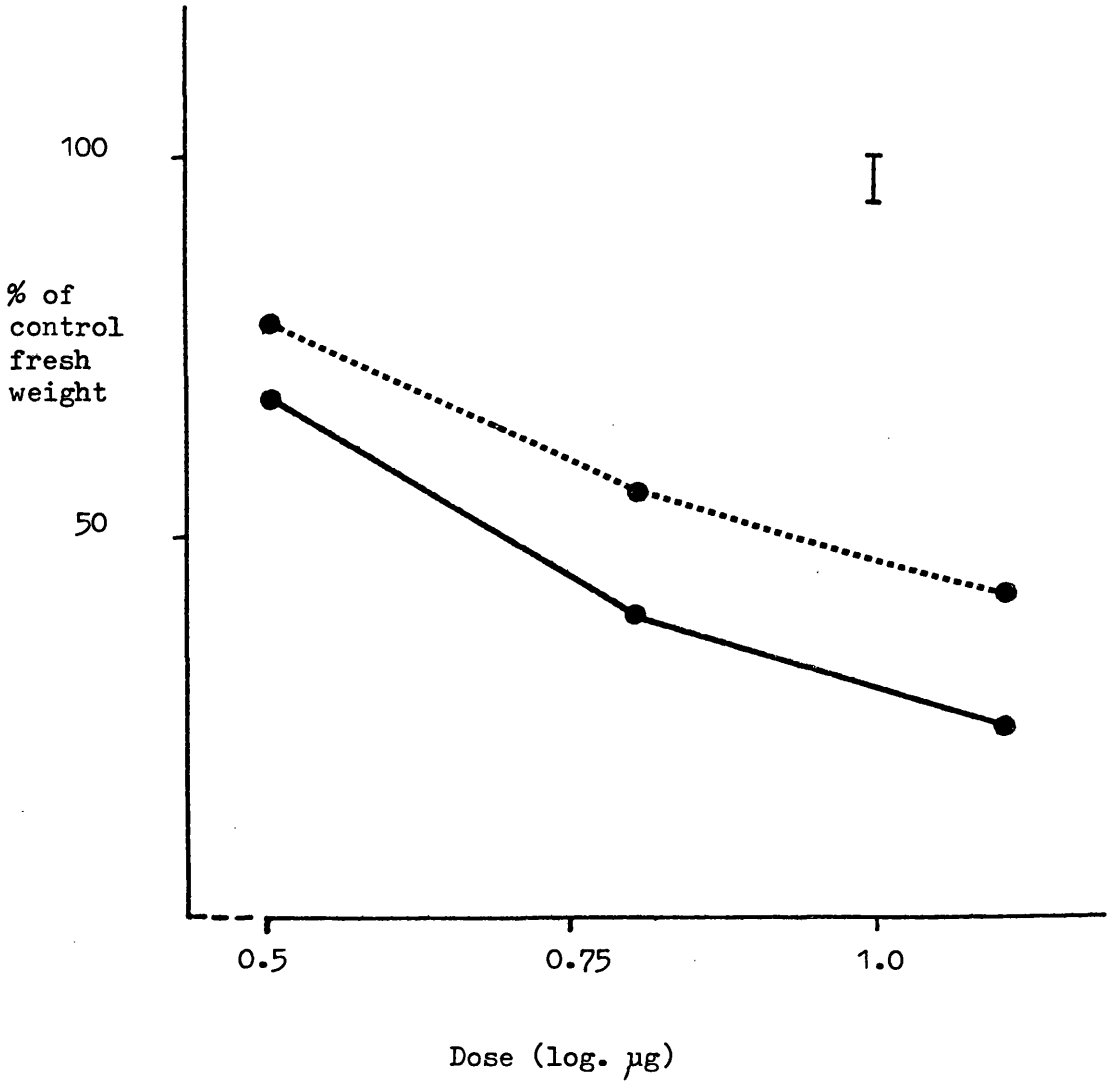


Figure 4.21: The response of radish (3 - 4 foliar leaves) to paraquat at three doses and two concentrations, using 300 µm drops.

Concentrations: 14 gl^{-1} .....

112 gl^{-1} ——

doses applied were again 3.2, 6.4 and 12.7 μg placed on the first pair of foliar leaves as 300 μm drops. There were fifteen replicates and fresh and dry weights were determined 11 days after treatment.

In this experiment there were no significant differences between the biological response to the concentrations used, despite highly significant dose responses ($p < 0.001$) for both fresh and dry weights. Figure 4.22 shows the results for fresh weight, those for dry weight being almost identical. Results for fresh weight : dry weight showed no significant differences, even for dose response.

The results for these two experiments show that increasing the concentration of paraquat and applying fewer drops causes no loss in paraquat effect, indeed the first experiment suggests a possible enhancement at concentrations in the region of 112 $g.l^{-1}$. It is possible that the difference between the two experiments in showing enhancement was related to the environmental conditions at the time of treatment since paraquat movement and effect are known to be dependent on environmental factors, particularly light (Dodge, 1971). The suggestion of an enhanced effect of paraquat at higher concentration is apparently contradictory to the usual assumption that this herbicide shows limited movement following application. Other such herbicides are generally more effective when applied in a more dispersed manner over the target surface. The results also contrast with those obtained in this study with difenzoquat on wild oat. Thus although necrotic symptoms appear very rapidly with paraquat at the site of treatment this does not seem to reduce effectiveness in the same way that such damage reduces difenzoquat performance, possibly due to the much more rapid entry of paraquat.

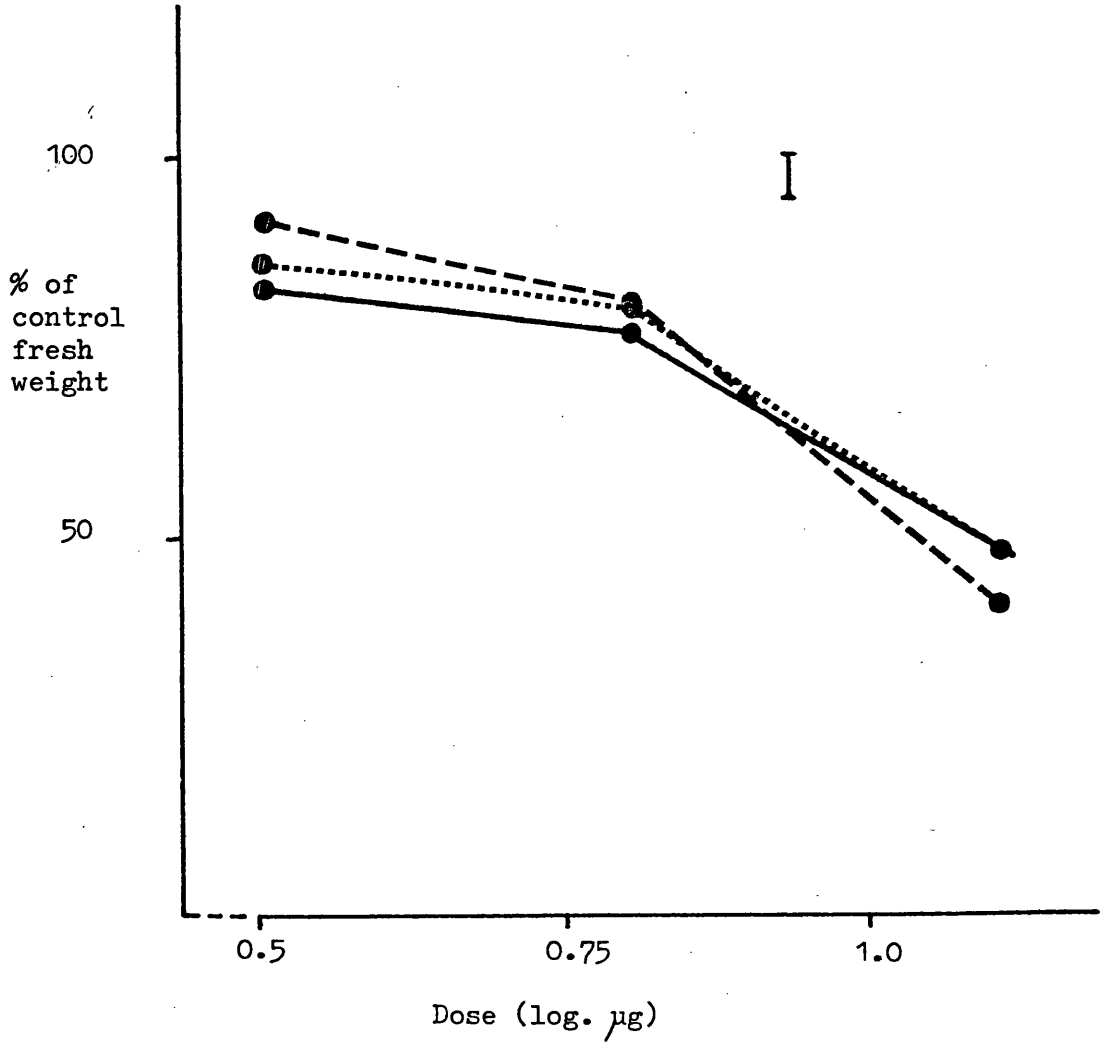


Figure 4.22: The response of radish (2 - 2½ leaves) to paraquat at three doses and three concentrations, using 300 µm drops.

Concentrations: 14 gl^{-1} .....

28 " – – –

56 " ———

4.3.3.3 The effect of drop size. In two similar experiments three drop sizes (200, 318, 400 μm) were applied in appropriate numbers to give 3.75, 7.5 and 15.0 μg of paraquat cation per plant using a concentration of 28 $g.l^{-1}$ of paraquat. In both experiments the radish plants had 2 fully expanded foliar leaves to which the drops were evenly applied. Assessment of fresh weight was made 16 days after treatment with the first experiment and both fresh and dry weight were assessed after 13 days in the second experiment.

The results for the first experiment showed a shallow, though significant ($p = 0.05$) dose response (Figure 4.23). There was no significant difference between drop sizes. The results of the fresh weight assessment in experiment 2 were identifical to those for dry weight, and are presented in Figure 4.24. Dose response was highly significant in this experiment ($p < 0.001$) but again there were no differences between drop sizes. These results are not substantially different from those of Douglas (1968) who, using the more dilute solutions of 0.9 - 7.5 $g.l^{-1}$ paraquat cation, found that an increase in drop size from 250 μm to 450 μm actually caused a slight increase in activity on broad bean, although above about 500 μm activity decreased with further increases in drop size. Buchring et al, (1969) also found the optimum drop size for paraquat to be around 473 μm on Cotton. McKinlay et al, (1974) found 100 μm drops to be more effective than 350 μm using sunflower as the test species and paraquat concentrations of 1.6 - 25.5 $g.l^{-1}$. However these were sprayed experiments and as such will also be influenced by differences in retention and position of the retained spray on the plant. These factors in turn are influenced by formulation, particularly surfactant concentration.

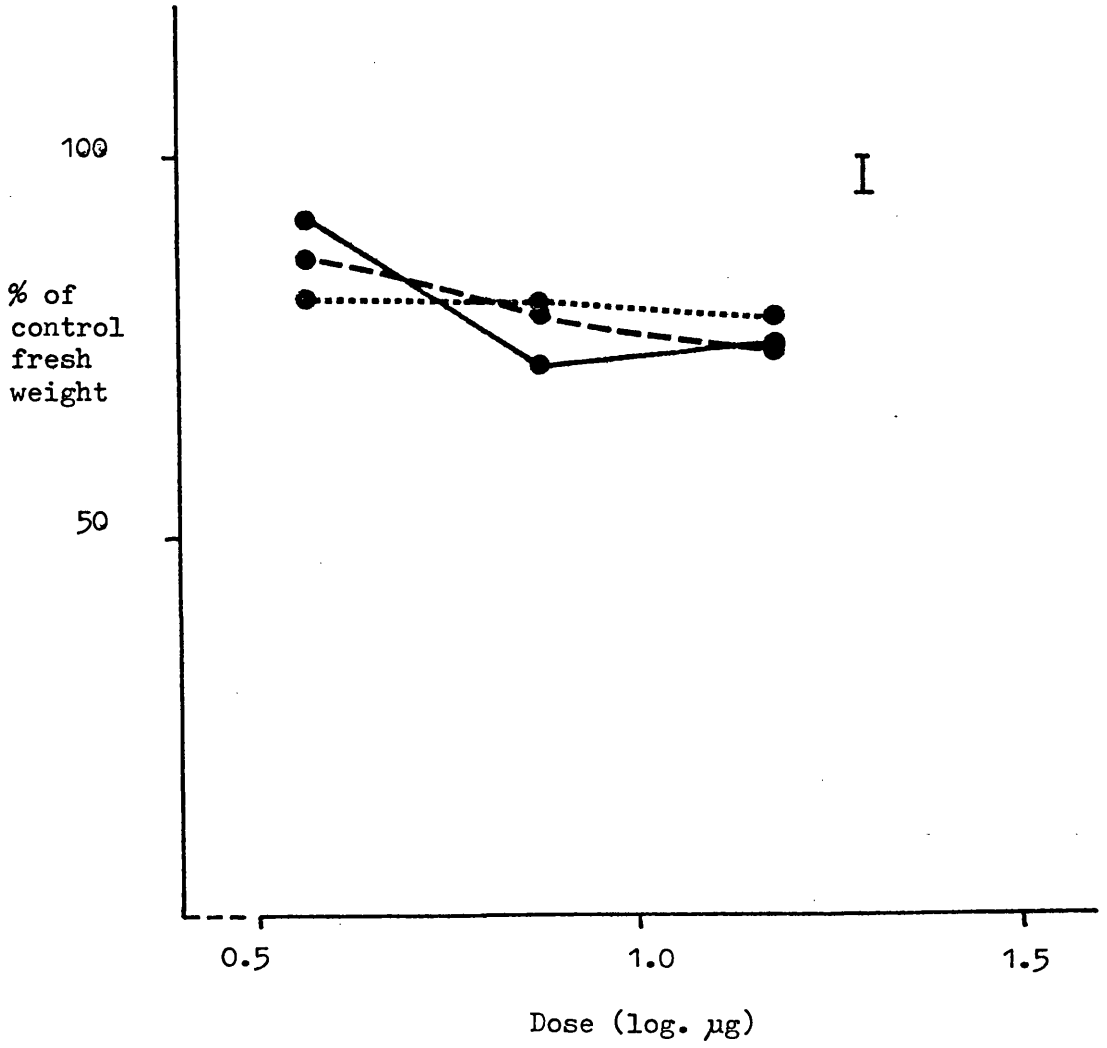


Figure 4.23: The response of radish (2 foliar leaves) to paraquat at three doses and three drop sizes, using a concentration of 28 gl^{-1} (First experiment)

Drop sizes: 200 µm .....

318 µm – – –

400 µm ———

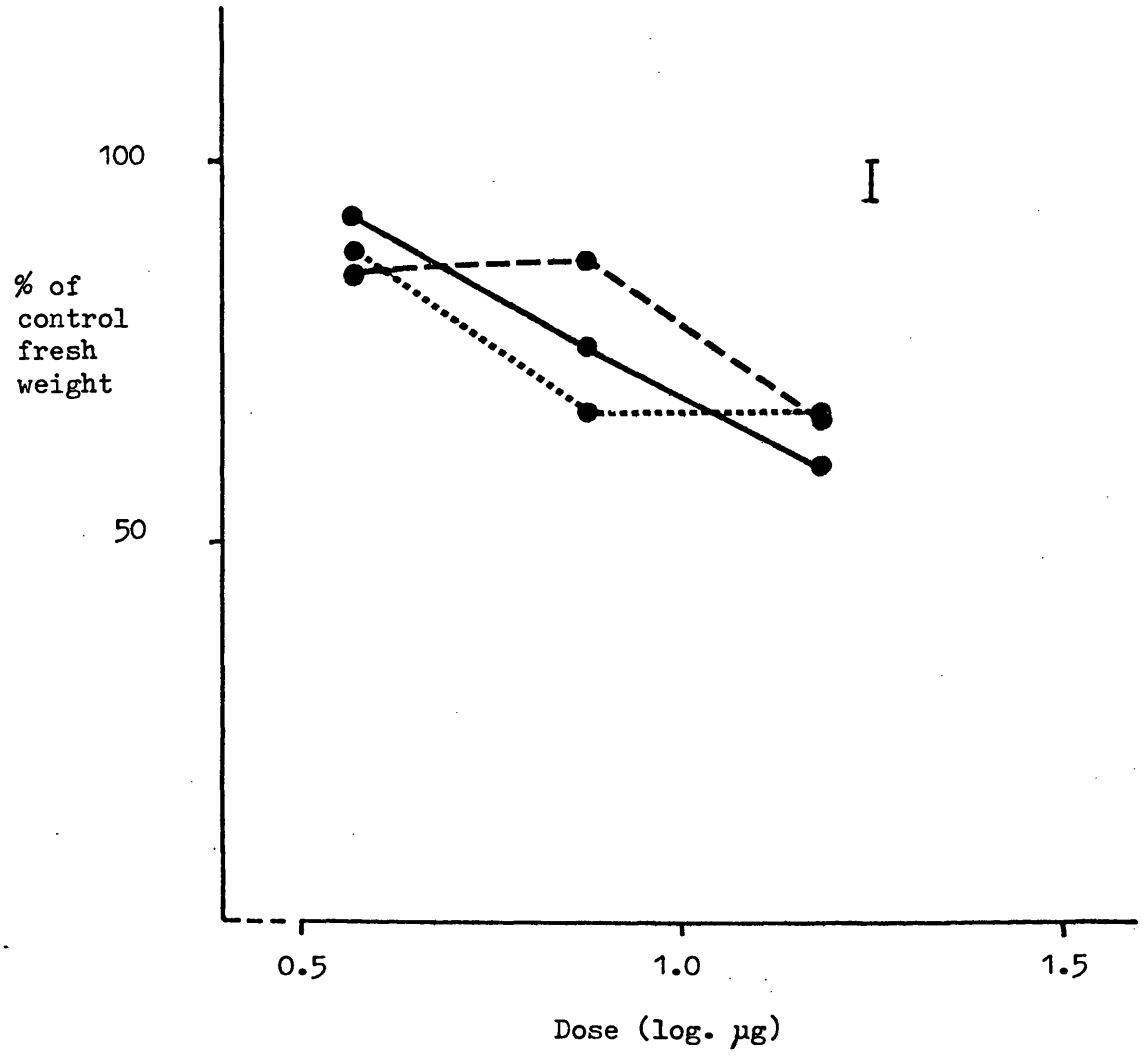


Figure 4.24: The response of radish (2 foliar leaves) to paraquat at three doses and three drop sizes, using a concentration of 28 gl^{-1} (Second experiment)

Drop sizes: 200 µm .....

318 µm – – –

400 µm ———

4.3.3.4 The effect of position of deposit. Three experiments were conducted to study the effects of varying the position of deposit on paraquat performance.

A first experiment compared treatment of the cotyledons with treatment of the first pair of foliar leaves and treatment of both cotyledons and foliar leaves, using plants with 2-2½ leaves. A solution of 28 $g.l^{-1}$ paraquat cation was used to apply 6.4, 12.7 and 25.5 µg as 300 µm drops. Fresh and dry weight were determined after 13 days.

The results for fresh weight (Figure 4.25) and dry weight were similar, showing a highly significant dose response ($p<0.001$) but no significant positional differences. However, there was a consistently greater reduction in both fresh and dry weights with treatment of the cotyledons compared with the foliar leaves, and in this experiment calculation of fresh weight : dry weight ratio showed this effect of position to be significant (Figure 4.26). This again shows that the best method of assessment may vary between experiments and since a high general dose response was recorded here the ratio of fresh to dry weight was the most useful criterion.

The observation here that treatment of the cotyledons was more effective than treatment of the foliar leaves, with a split application to both coming somewhere between, is contrary to the result obtained with MCPA in an otherwise similar experiment (Section 4.3.1.4). This may be indicative of differences in the entry and movement of the two herbicides. MCPA is known to be well translocated and may well follow the assimilate translocation system out of the leaves (Crafts and Crisp, 1971), whilst paraquat is thought to move predominantly in the apoplast, following the movement of water, and thus may benefit from placement lower down the plant, since the transpiration stream

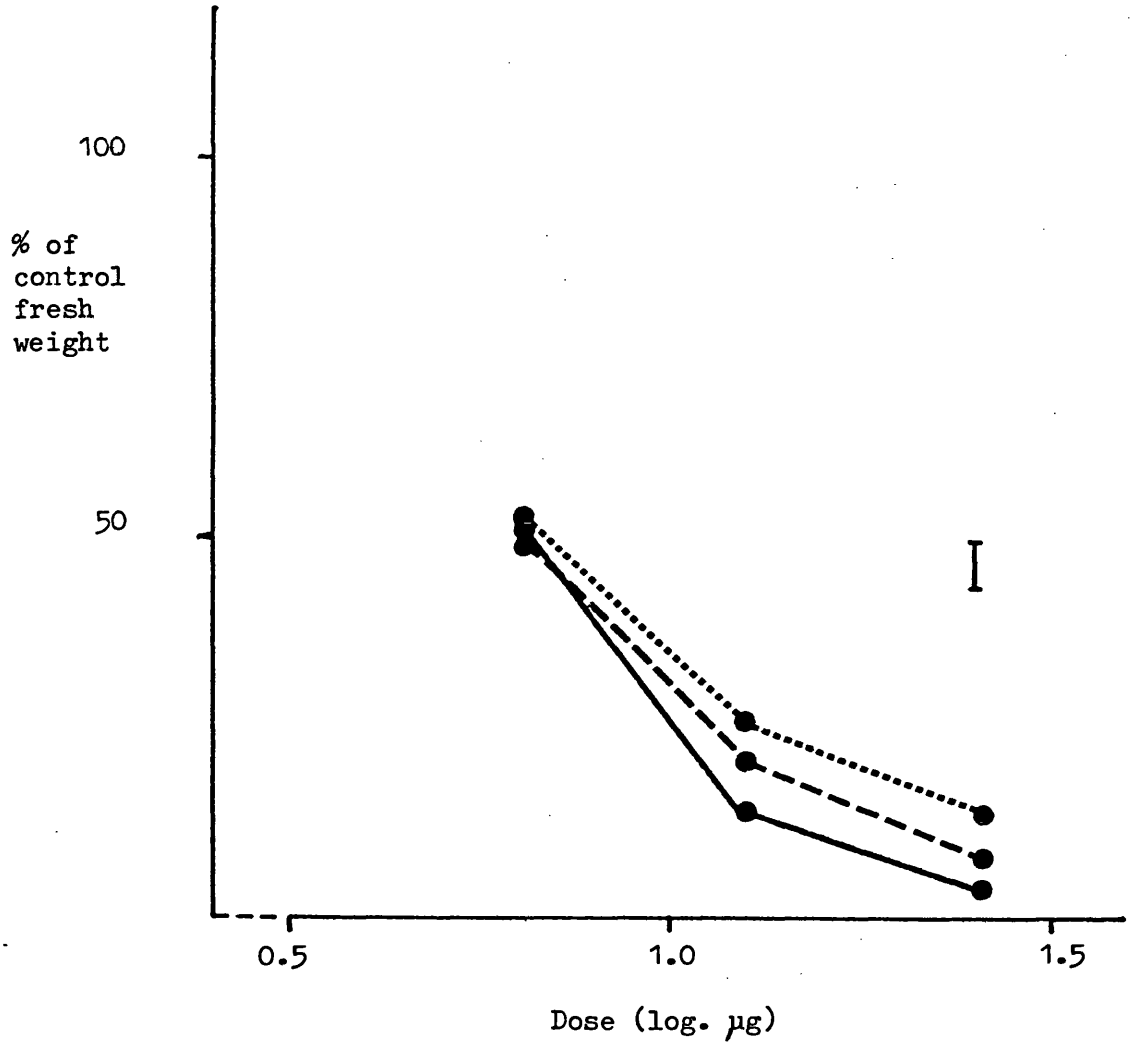


Figure 4.25: The response of radish (2 - 2½ foliar leaves) to paraquat at three doses using a concentration of 28 gl^{-1} and 300 µm drops, applied to three positions: fresh weight assessment.

Positions: Foliar leaves only .....

Cotyledons only - - -

Foliar leaves and cotyledons ——

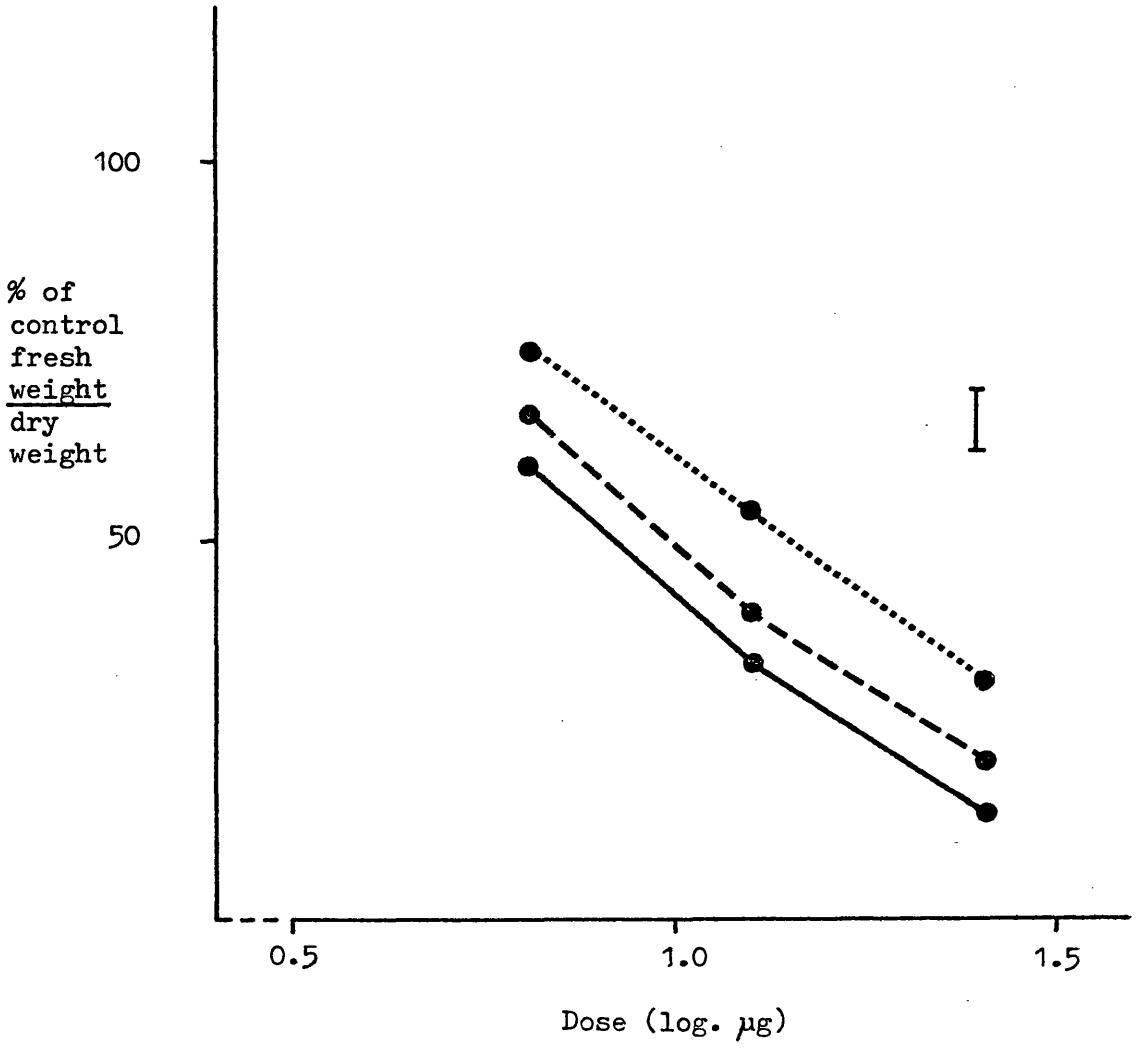


Figure 4.26: The response of radish (2 - 2½ foliar leaves) to paraquat at three doses using a concentration of 28 gl^{-1} and 300 µm drops, applied to three positions; fresh weight/dry weight assessment.

Positions: Foliar leaves only .....

Cotyledons only ——

Foliar leaves and cotyledons – – –

will tend to aid movement towards the foliar leaves. It is also noticeable that the surfaces of the cotyledons and foliar leaves differ considerably, and it is also probable that the mechanism of entry differs between these chemically different herbicides. In addition the penetration of paraquat (Hunyadi, 1973) appears to occur much more rapidly than that of MCPA (Kirkwood et al, 1972), highlighting differences between these compounds.

A second experiment was conducted using plants with two fully expanded foliar leaves. Using the same concentration of paraquat as in the previous experiment, a dose of 15 μg as sixteen 400 μm drops, applied to the second pair of foliar leaves, was compared with three doses of 3.8, 7.5 and 15.0 μg applied to the first pair of foliar leaves, also as 400 μm drops. At the time of treatment the second pair of foliar leaves were just emerging, and presented a leaf area estimated as about one eighth that of the first pair.

A fresh weight assessment after 13 days showed that treatment of these young leaves was much less effective than treatment of the mature leaves (Figure 4.27). As with the results of the previous experiment this could be associated with the relationship of the site of treatment to the accessibility of the pathways of water movement in the plant.

In the third experiment on position of deposit paraquat (28 $g.l^{-1}$) was applied as 3.2, 6.4 and 12.7 μg using 300 μm drops to either the midvein or the regions of lamina between major veins on the foliar leaves of plants with 2 fully expanded foliar leaves. Fresh weight and dry weight were assessed after 14 days.

There was a significant dose response for both assessments

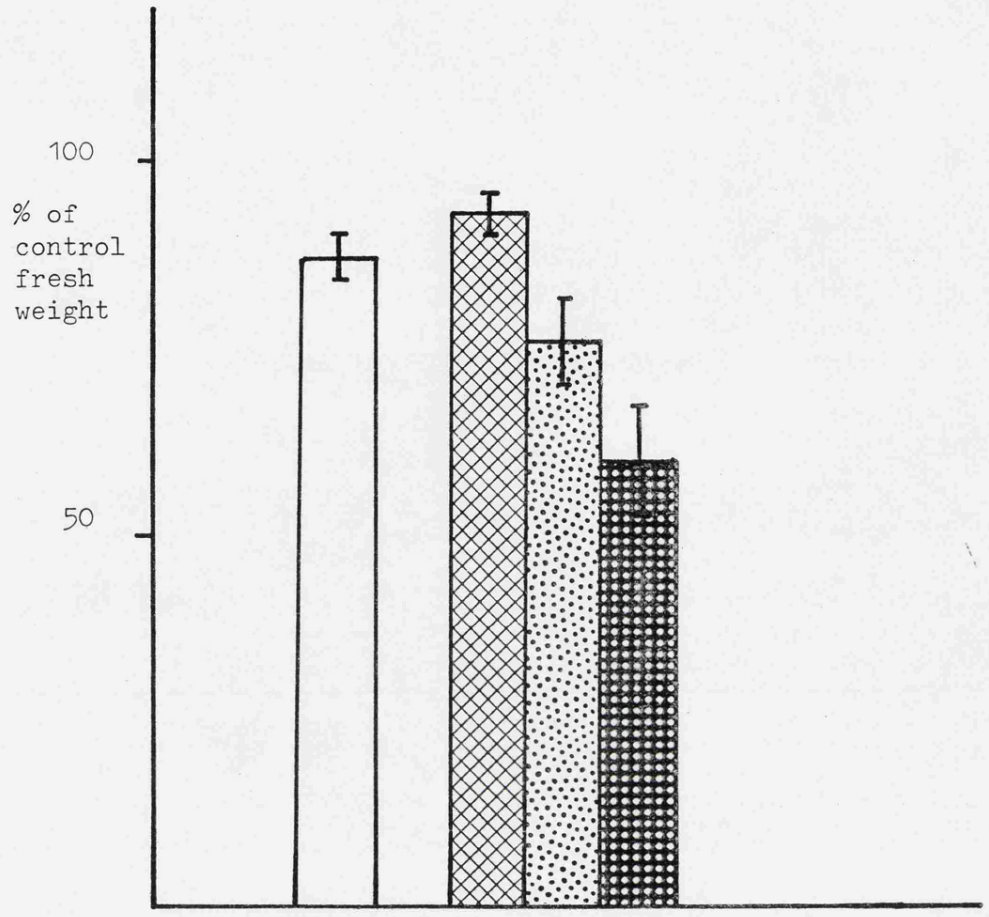


Figure 4.27: The response of radish (2 leaves) to paraquat applied to two positions.

15 µg paraquat, applied to young leaves

3.8 µg paraquat, applied to expanded leaves

7.5 µg paraquat, applied to expanded leaves

15 µg paraquat, applied to expanded leaves

($p = 0.05$) and highly significant differences between positions ($p < 0.001$ for fresh and $p = 0.01$ for dry weight). The results for fresh weight (Figure 4.28) showed that treatment of the midvein was at all doses more effective than treatment of the lamina between veins. This is consistent with the idea that paraquat movement and effect relies on access to the water movement pathways, which occur abundantly in the veins. A similar result occurred with MCPA (See Section 4.3.1.4) but as previously stated the midvein is more readily wetted than the rest of the lamina, so access to water movement pathways may not be the only factor involved.

4.3.3.5 The effect of surfactant concentration. Plants with 2-3 foliar leaves were treated using 28 $g.l^{-1}$ paraquat solution containing 0.01, 0.1 or 1.0% v/v Agral surfactant. Doses of 6.4, 12.7 and 25.5 µg were applied as 300 µm drops to the first pair of foliar leaves. Fresh weight was assessed after 12 days.

The results (Figure 4.29) show a highly significant dose response ($p < 0.001$) but no significant differences between surfactant concentrations. There is little previous published data on the effects of surfactant concentration on paraquat activity, although Smith, Foy and Bayer (1966) found that paraquat effects on corn increased with surfactant concentrations in the range 1-100 $g.l^{-1}$. However their experiments were sprayed and the effects of varying surfactant concentration on retention of spray by the foliage cannot be discounted. Bland and Brian (1975) concluded that paraquat movement could be affected by concentrations of surfactants in the range 0.1 - 0.5% concentration but no such effect was seen in these experiments.

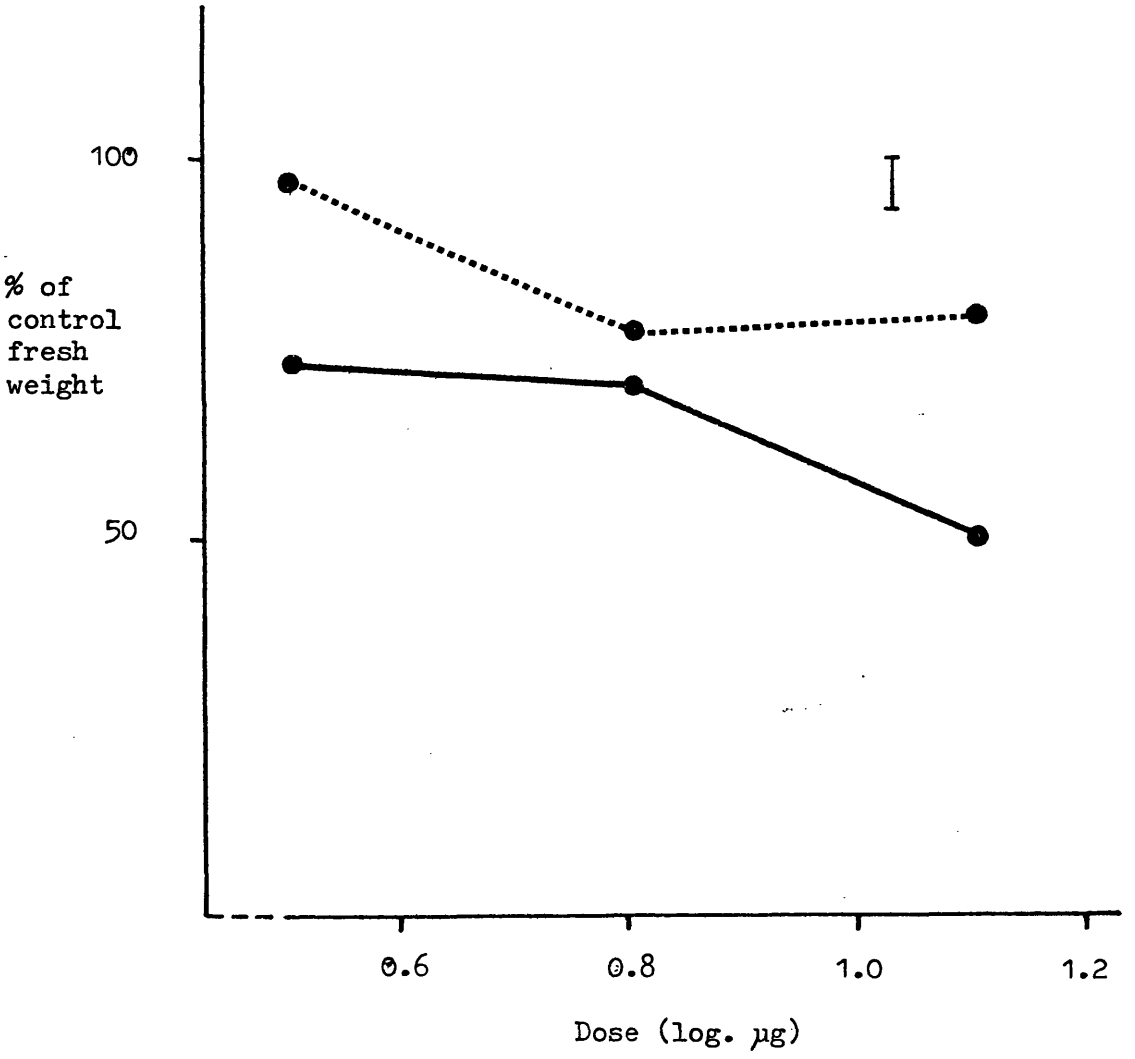


Figure 4.28: The response of radish (2 foliar leaves) to paraquat at three doses using a concentration of 28 gl^{-1} and 300 µm drops, applied to two positions.

Positions: Midvein ——

Lamina between veins ·····

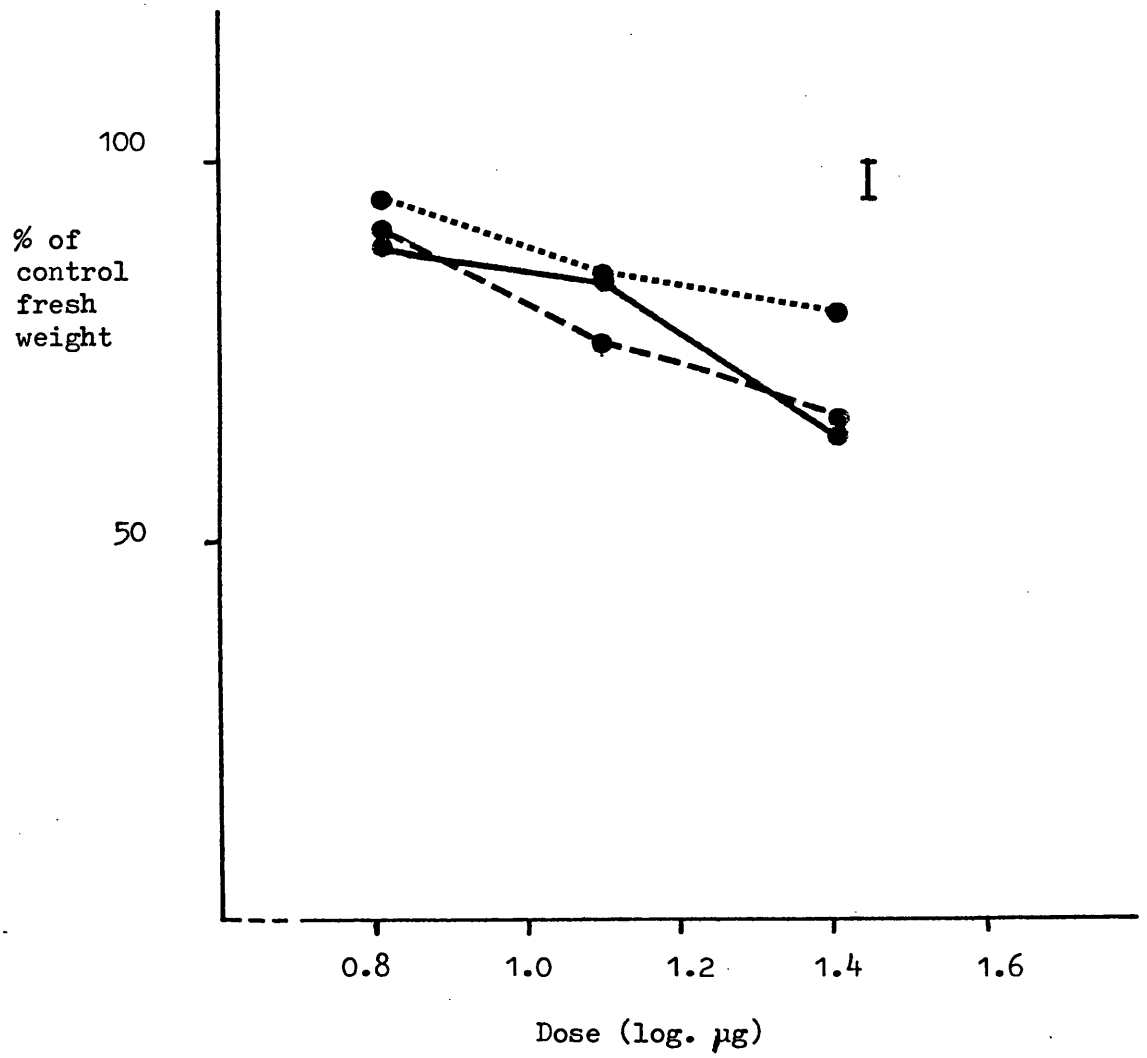


Figure 4.29: The response of radish (2 - 3 foliar leaves) to paraquat at three doses and a concentration of 28 gl^{-1} using three concentrations of 'Agral' surfactant.

'Agral' concentrations 0.01 % v/v .....

0.1 % v/v – – –

1.0 % v/v ——

4.3.4 Experiments with paraquat on wild oat

4.3.4.1 The effect of concentration. The dose response for paraquat on wild oat was determined approximately in a preliminary experiment of insufficient replication for analysis. A subsequent experiment investigated the effects of paraquat concentration. Plants with 3-3½ leaves were treated using 300 µm drops at dose levels of 0.4, 0.8 and 1.6 µg. Four concentrations were used, namely 3.5, 7.0, 14.0 and 28.0 $g.l^{-1}$ of paraquat, with Agral concentration maintained at 0.1% v/v throughout; There were ten replicates. The treatments were applied to the second leaf 20-40 mm from the ligule and fresh weight was determined after 18 days.

The level of response was unexpectedly high (Fig. 4.30) and this may have reduced chances of detecting treatment differences, although the dose response was highly significant ($p<0.001$). The clearest differences might have been expected at the lowest dose but no clear trend or significant effect of concentration was apparent. The most striking feature was indeed the close agreement between the highest and lowest concentrations, these being an eightfold difference in concentration and drop number. This is generally similar to the findings with radish given in Section 4.3.3.2. As with paraquat on radish the observed effect of the treatments suggests considerable movement of the herbicide to non-treated parts, or at least movement of a toxic amount of herbicide. Perhaps more surprisingly, these results are unlike those obtained with difenzoquat on wild oat (Section 4.3.2.2) where activity decreased with increasing concentration. It was observed that paraquat treated leaves began to wilt very rapidly after treatment (within 1-2 hours) suggesting that paraquat may disperse locally (around the site of treatment) more rapidly than difenzoquat and perhaps

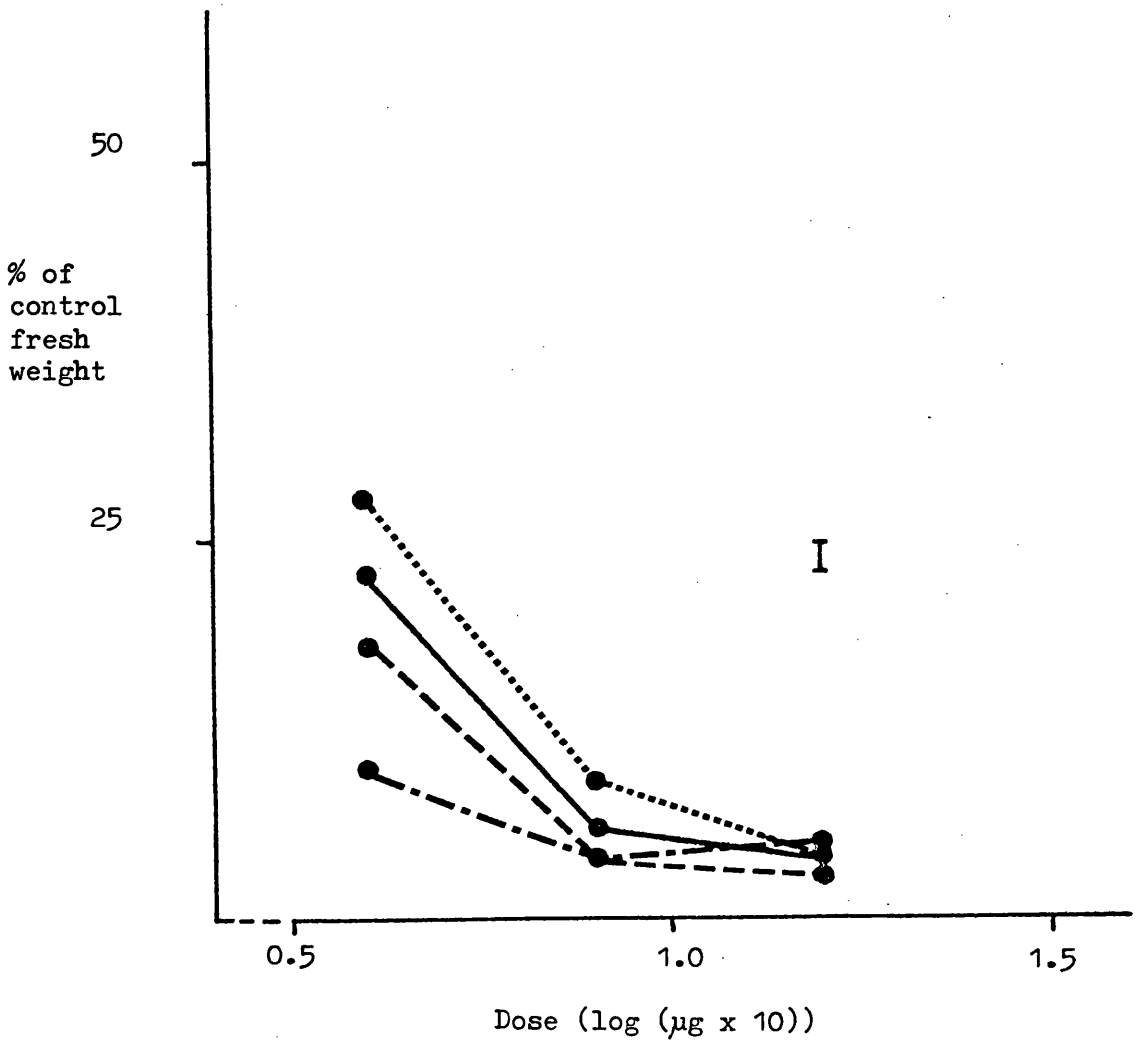


Figure 4.30: The response of wild oat (3 - 3½ leaves) to paraquat at three doses and four concentrations, using 300 µm drops

Concentrations: 3.5 gl^{-1} .....

7 " – – –

14 " – · –

28 " ———

thereby avoiding becoming isolated in dead or dying tissue as appears to be the case with difenzoquat.

4.3.4.2 The effect of drop size. Plants with three leaves were treated on the second leaf 20-40 mm from the ligule. Paraquat solution containing 28 $g.l^{-1}$ was used to apply three doses of 0.94, 1.88 and 3.75 μg as three drop sizes of 200, 318 and 400 μm diameter. Eight replicates were treated in this experiment and fresh weight was assessed after 19 days.

The results are presented in Figure 4.31 and show no significant effect of drop size, despite a highly significant dose response ($p = 0.01$). This result is in agreement with those using radish (Section 4.3.3.3) and also with a laboratory experiment using wild oats sprayed in a cabinet at drop sizes between 150 and 350 μm with a controlled drop size sprayer (Merritt and Taylor, 1978, unpublished work).

4.3.4.3 The effect of position of deposit. Two experiments were conducted to investigate the difference in sensitivity between the leaves of different age on three-leaf wild oats. The experiments differed in that glasshouse-grown plants were used in the first and outdoor grown plants in the second. In both experiments doses of 0.4, 0.8 and 1.6 μg were applied as 1, 2 or 4 drops of paraquat solution containing 28 $g.l^{-1}$ cation and 0.1% v/v Agral. The doses were applied to the leaf lamina 20-40 mm from the ligule on leaves 1, 2 and 3. There were six replicates in the first experiment and ten in the second; fresh weight was determined after 18 and 12 days respectively.

The results are presented in Figure 4.32 (first experiment) and 4.33 (second experiment). In both experiments there was a highly

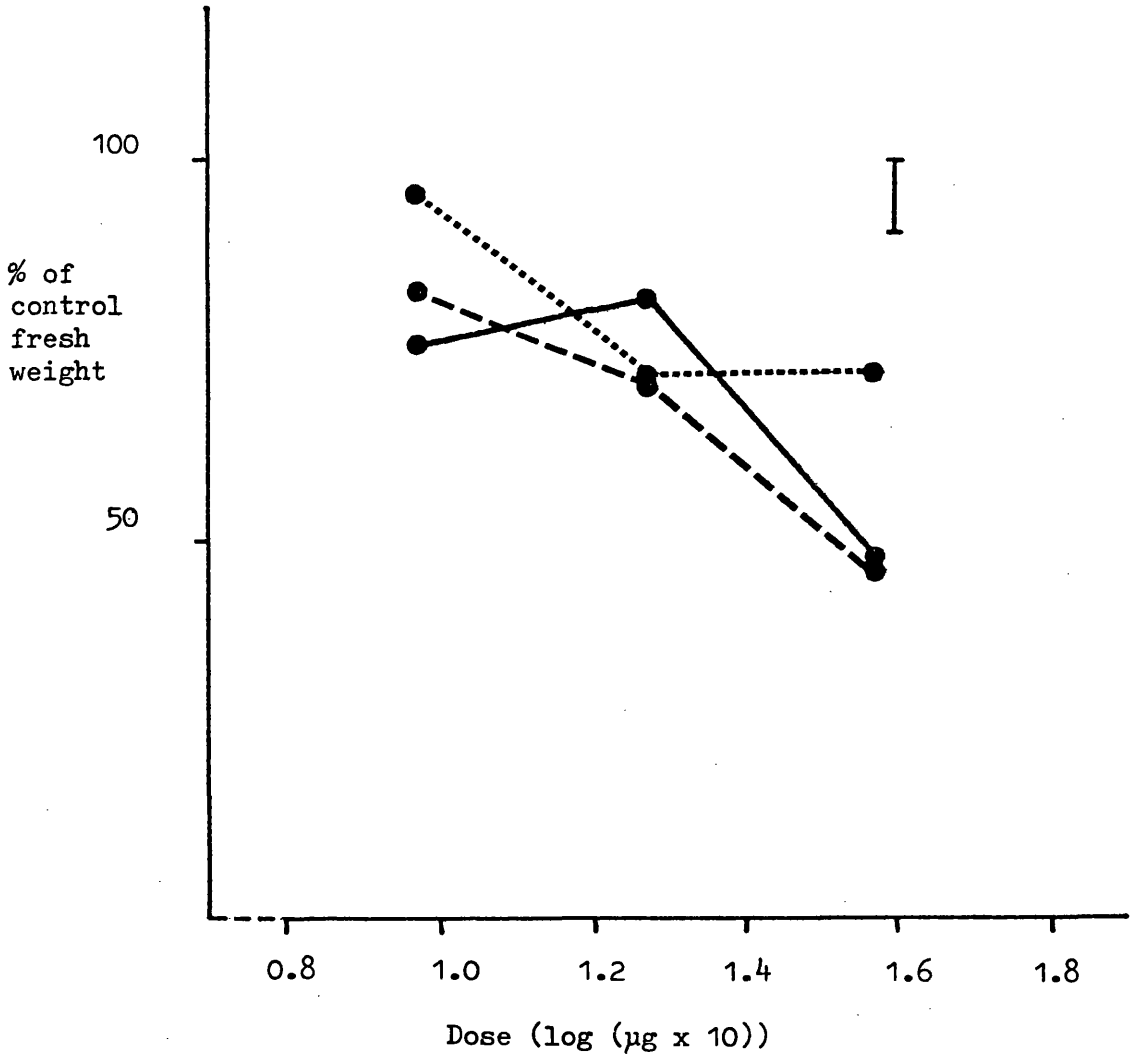


Figure 4.31: The response of wild oat (3 leaves) to paraquat at three doses and three drop sizes using a concentration of 28 gl^{-1}

Drop sizes: 200 µm .....

318 µm – – –

400 µm ———

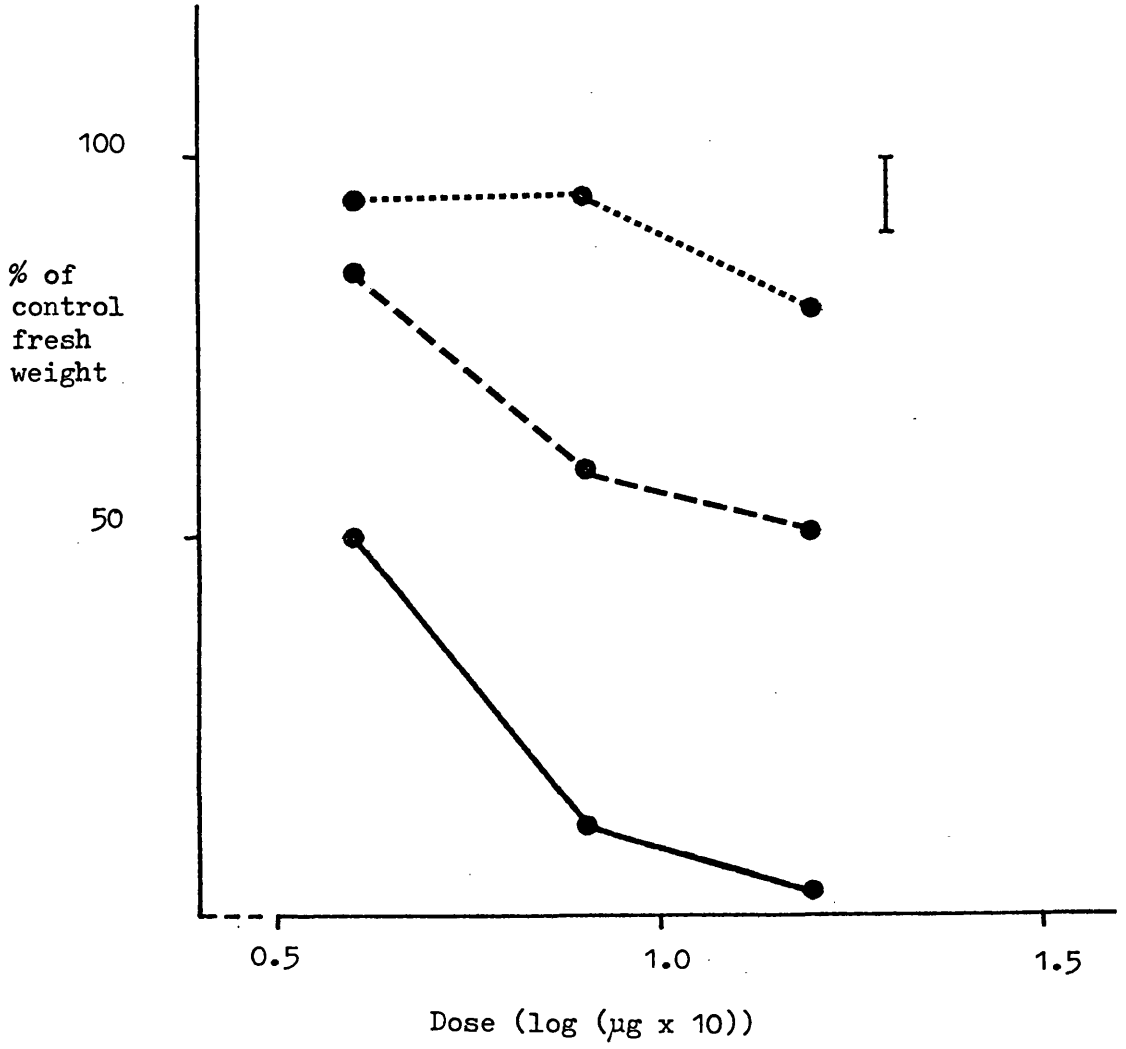


Figure 4.32: The response of wild oat (3 leaves) to paraquat at three doses using a concentration of 28 gl^{-1} and 300 µm drops, applied to three positions (First experiment)

Positions: Leaf 1 .....

Leaf 2 – – –

Leaf 3 ——

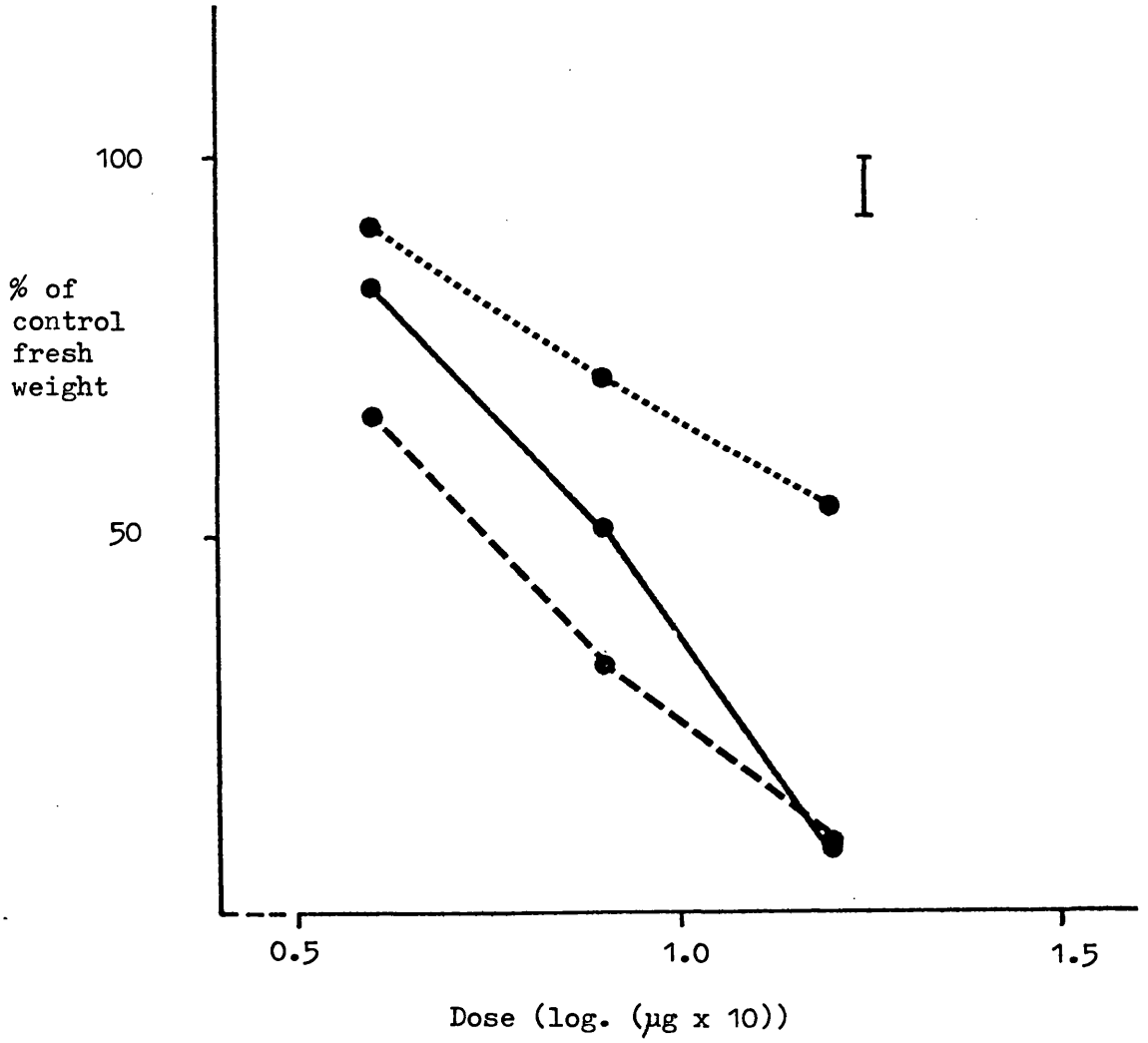


Figure 4.33: The response of wild oat (3 leaves) to paraquat at three doses using a concentration of 28 gl^{-1} and 300 µm drops, applied to three positions (Second experiment)

Positions: Leaf 1 .....

Leaf 2 – – –

Leaf 3 ———

significant effect of both dose ($p = 0.01$ in experiment 1 and $p < 0.001$ in experiment 2) and position of deposit ($p < 0.001$ in both experiments). Also in both experiments the least effective position at all doses was that of leaf 1. There is a discrepancy in the relative effectiveness of leaves 2 and 3 in that leaf 3 was the most effective position in the first experiment and leaf 2 in the second. However the general conclusion that effectiveness as site of treatment is reduced on older leaves agrees well with the work using difenzoquat (Section 4.3.2.4) and with the work of Coupland, Taylor and Caseley (1978), who suggest that the observed differences may be due to the metabolic condition of the leaf, in particular whether the translocation of assimilates is occurring into or out of the leaf and the degree of senescence. Observations on herbicide symptoms in these experiments would seem to add support to this suggestion; it was apparent that chlorosis and subsequently necrosis appeared in the young leaves which emerged after treatment, and that these symptoms were more severe when younger leaves were treated.

4.3.5 Experiments with glyphosate on radish

4.3.5.1 The dose response. A range of doses of glyphosate (4.1 - 65.4 µg a.e.) was applied to radishes with two fully expanded foliar leaves and a mean dry weight of 0.14 g per plant. The doses were applied as 4-64 drops of 300 µm diameter of a solution containing 72 $g.l^{-1}$ glyphosate acid equivalent and 0.1% v/v 'Agral'. Drops were spread evenly over the foliar leaves. Ten replicates were treated and fresh and dry weights were assessed 19 days after treatment.

A significant dose response (See Figure 4.34) was recorded for fresh weight, dry weight and fresh weight : dry weight ratio

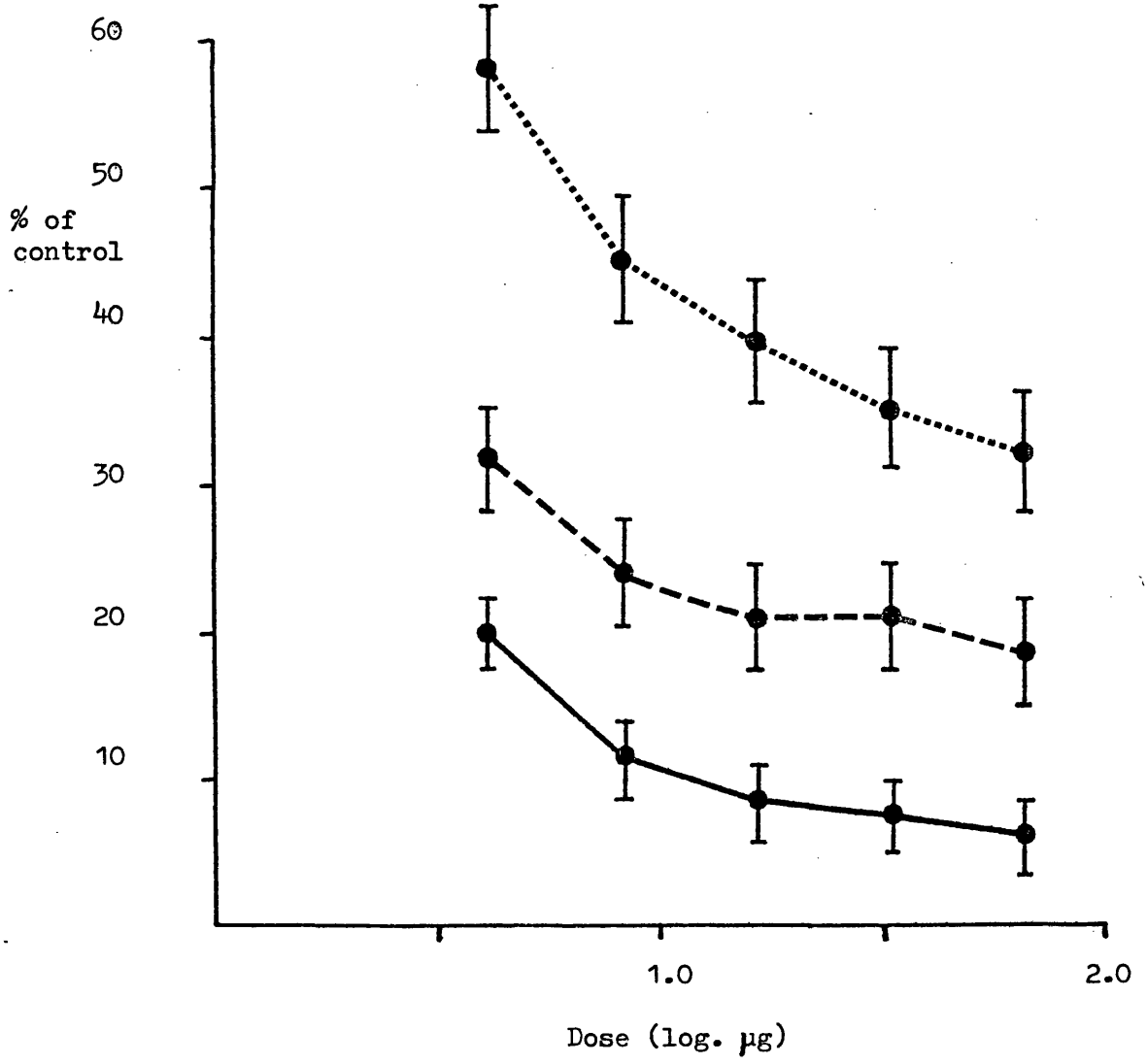


Figure 4.34: The response of radish (2 leaves) to a range of doses of glyphosate, applied as 300 µm drops using a concentration of 72 gl^{-1} a.e.

Assessment: **Fresh weight**

Dry weight

Fresh weight/Dry weight

($p<0.001$ for all three analyses). The radishes showed typical symptoms of glyphosate treatment with no visible effects for the first few days followed by slowly developed chlorosis with some red pigmentation developing. No marked effects were apparent at the site of drops. No plants were killed at the lowest two doses, whilst two out of ten plants were killed by 8.2 μg rising to eight plants killed by 65.4 μg. Thus the optimum dose for subsequent experimentation appeared to be about 8.2 μg and just below that. Comparing the relative effects it would seem that fresh weight would be most useful at the lower doses since this showed the greatest reduction relative to the untreated plants.

4.3.5.2 The effect of concentration. The effect of glyphosate concentration was studied on plants with two foliar leaves and a mean fresh weight of 1.98 grams. Three doses of 2.05, 4.09 and 8.18 μg were applied using three concentrations of 36, 72 and 144 $g.l^{-1}$ a.e. Treatments comprised 300 μm drops applied evenly over the first pair of foliar leaves, and 15 replicates were used. Fresh and dry weights were determined 15 days after treatment.

Dose responses for fresh and dry weight were both significant ($p<0.001$) but with fresh weight : dry weight ratio there was no significant difference between treated plants and untreated controls. With both fresh and dry weight assessments there was a significant effect of concentration ($p<0.001$ in both cases) although the results differed slightly; with fresh weight all three concentrations were significantly different, with the most dilute solution being the least effective and the most concentrated solution the most effective (Figure 4.35), whilst with the dry weight assessment the two more concentrated solutions gave identical results, the most dilute solution

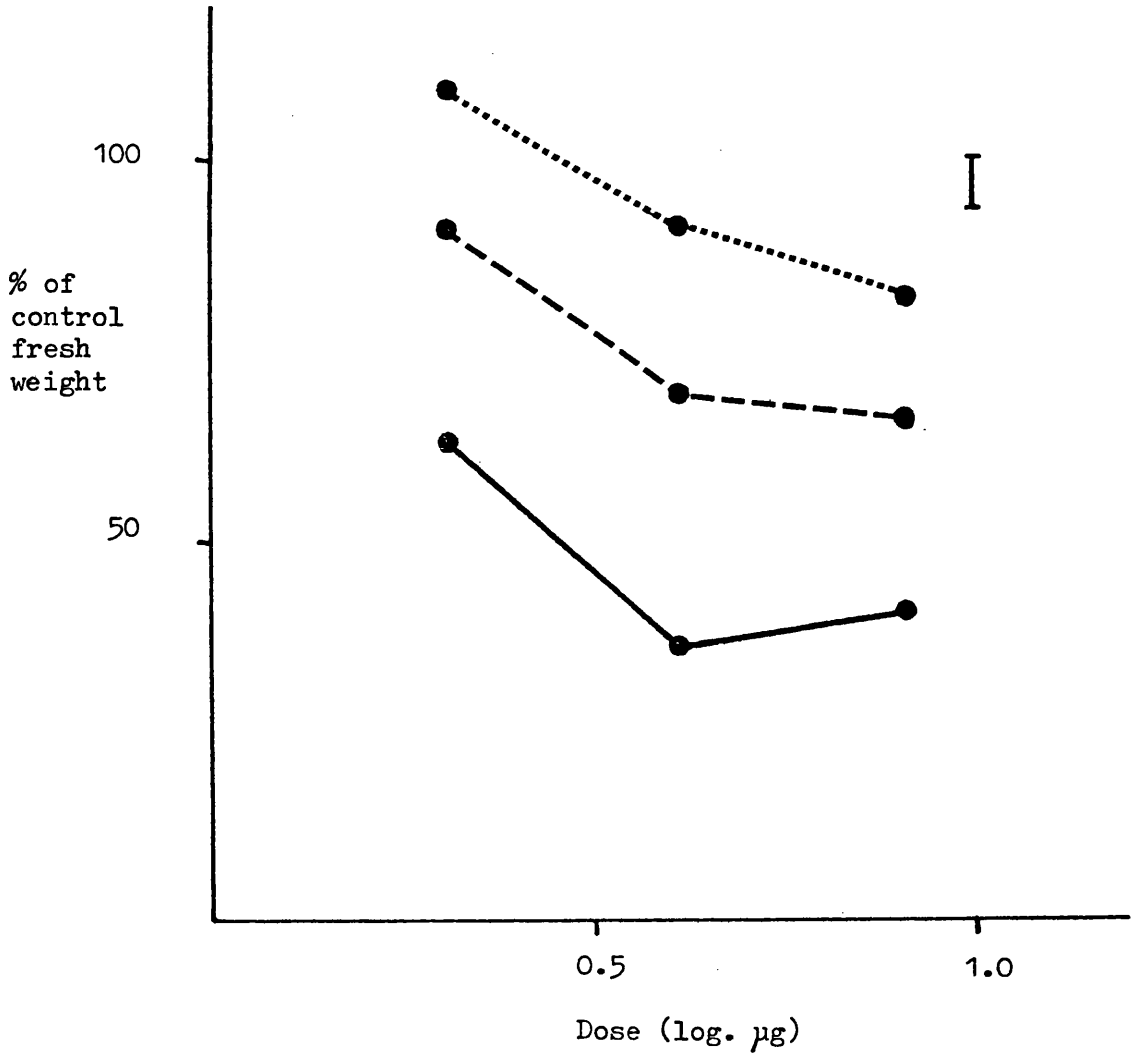


Figure 4.35: The response of radish (2 leaves) to glyphosate at three doses and three concentrations using 300 µm drops.

Concentrations: 36 gl^{-1} .....

72 " – – –

144 " ——

still being inferior to these (Figure 4.36). The results as a whole show that glyphosate is more effective on radish when applied as fewer drops of a more concentrated solution. This would suggest that improvements in glyphosate effectiveness that have been reported when applied at low volume rates (Turner and Loader, 1978) may be largely due to this concentration effect. It is not possible here to identify the cause of this effect, but it may be that a greater concentration gradient at the site of each drop increases the rate of entry and dispersion of the herbicide. It is also possible that confinement of glyphosate to discrete points on the leaf result in the remainder of the leaf maintaining a relatively healthy condition. Clearly this would be advantageous for the movement of glyphosate if this process depends on the efficient functioning of the phloem system and an abundant supply and export of assimilates. A notable feature of glyphosate symptoms on plants treated at very low volume rates is the lack of a local necrotic effect which is observed with many other herbicides, and in general symptoms are very slow to appear with this herbicide. Thus there is little likelihood that local disruption of tissue occurs to cause inhibition of movement away from the treated region, as was suggested for difenzoquat on wild oat (Section 4.3.2.2).

4.3.5.3 The effect of drop size. A solution containing 72 $g.l^{-1}$ glyphosate was applied to radishes with two foliar leaves using 200, 318 and 400 μm diameter drops. Numbers of drops were varied to give three doses of 2.05, 4.09 and 8.18 μg per plant for each drop size; and the drops were applied to the foliar leaves. Fresh weight was assessed 19 days after application.

There was a highly significant dose response ($p < 0.001$) shown with all drop sizes. Although there was no overall significant effect

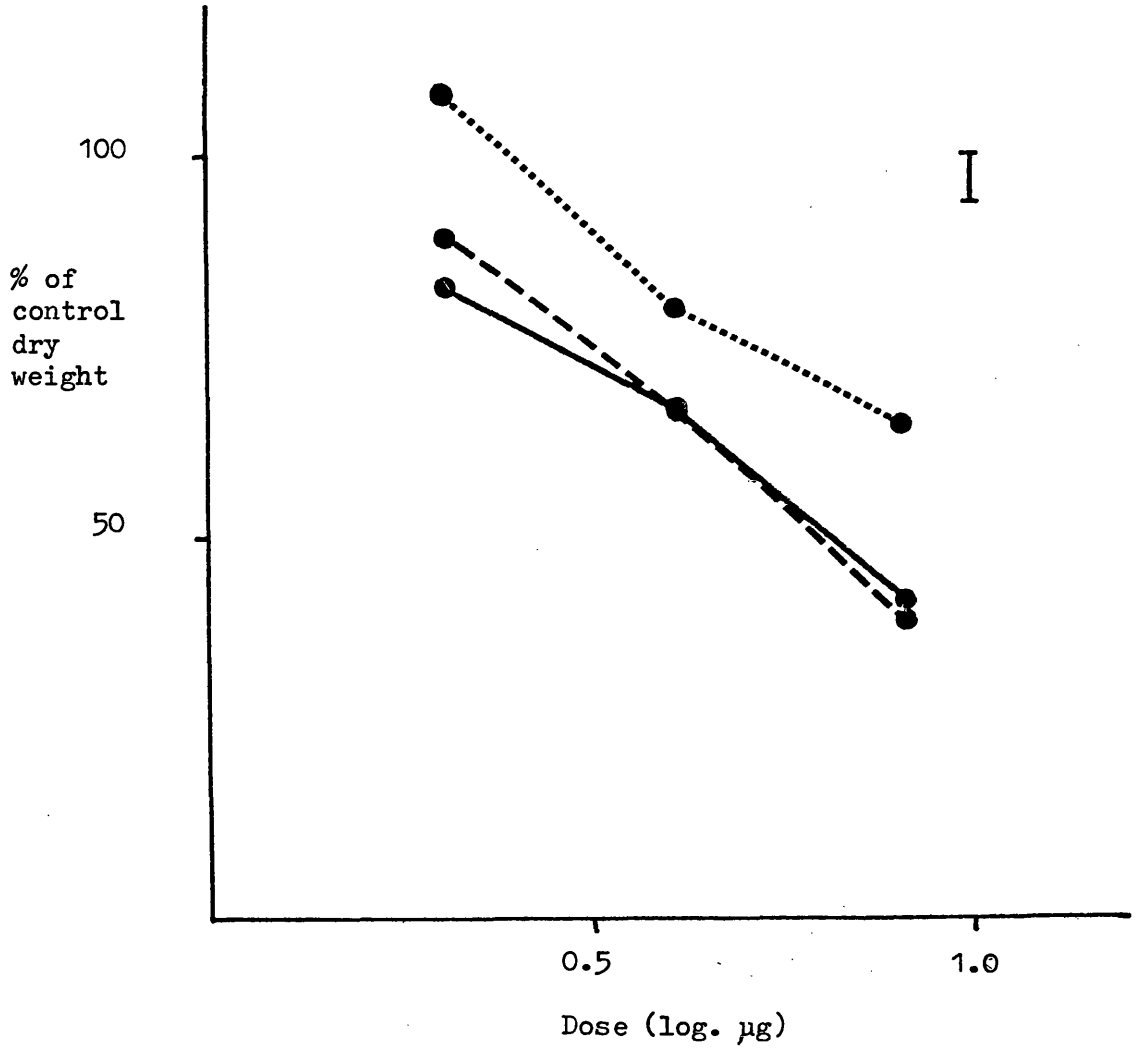


Figure 4.36: The response of radish (2 leaves) to glyphosate at three doses and three concentrations using 300 µm drops.

Concentrations: 36 gl^{-1} .....

72 " – – –

144 " ———

of drop size there was a significant interaction between drop size and dose ($p = 0.05$). This was due to the largest drop size (400 μm) which was more effective than the other drop sizes at the middle and higher dose but less effective at the lower dose (See Figure 4.37). The explanation for this effect was not clear from the available information, and it is tentatively concluded that there is little effect of drop size in the range 200-400 um on glyphosate performance on radish.

4.3.5.4 The effect of position of deposit. Radish plants with two foliar leaves were treated with three doses of 4.09, 8.18 and 16.4 μg glyphosate, using a solution containing 72 $g.l^{-1}$ and drops of 300 μm diameter. The treatments were applied to the cotyledons, the foliar leaves along the midvein, or to the areas of the foliar leaf laminae between veins. Fresh weight was assessed 19 days after application.

The results showed a highly significant dose response ($p = 0.001$) as shown in Figure 4.38. There was also a highly significant effect of position ($p < 0.001$) and a significant interaction of dose and position ($p = 0.01$). The interaction was due to treatment differences being lost at the lower dose where fresh weights of all treatments approached that of the untreated control. At the higher two doses interesting differences occurred between treatments. Treatment to the midvein of the foliar leaves was about as effective as treatment to the cotyledons. However, treatment to the foliar leaf laminae between veins was more effective than both the other positions. These results contrast with those obtained with both MCPA (Section 4.3.1.4) and paraquat (Section 4.3.3.4) since with these other herbicides the midrib was a more effective site of application than the leaf lamina. It is difficult to see where the difference lies but it

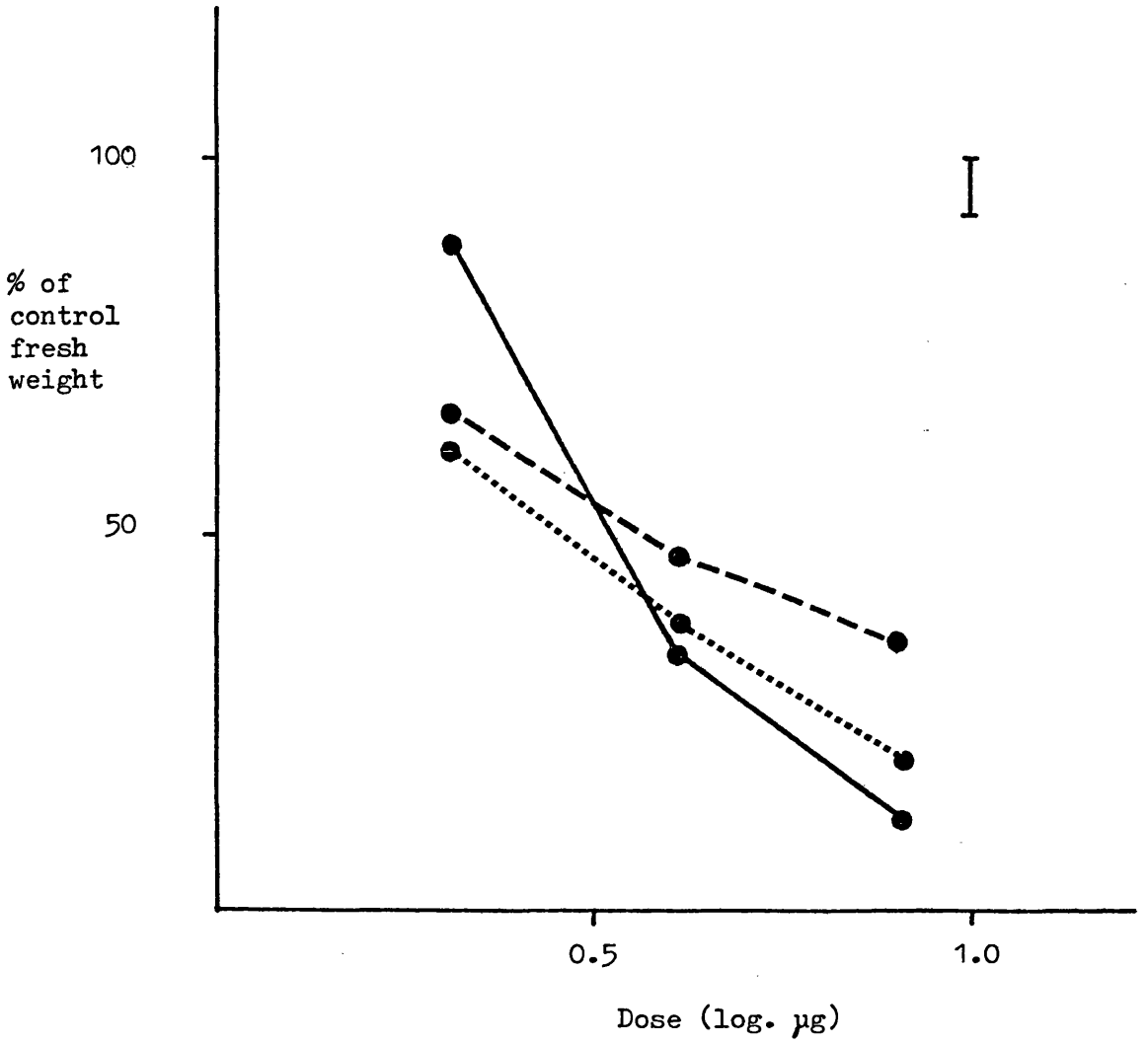


Figure 4.37: The response of radish (2 leaves) to glyphosate at three doses and three drop sizes using a concentration of 72 gl^{-1} a.e.

Drop sizes: 200 µm .....

318 µm – – –

400 µm ———

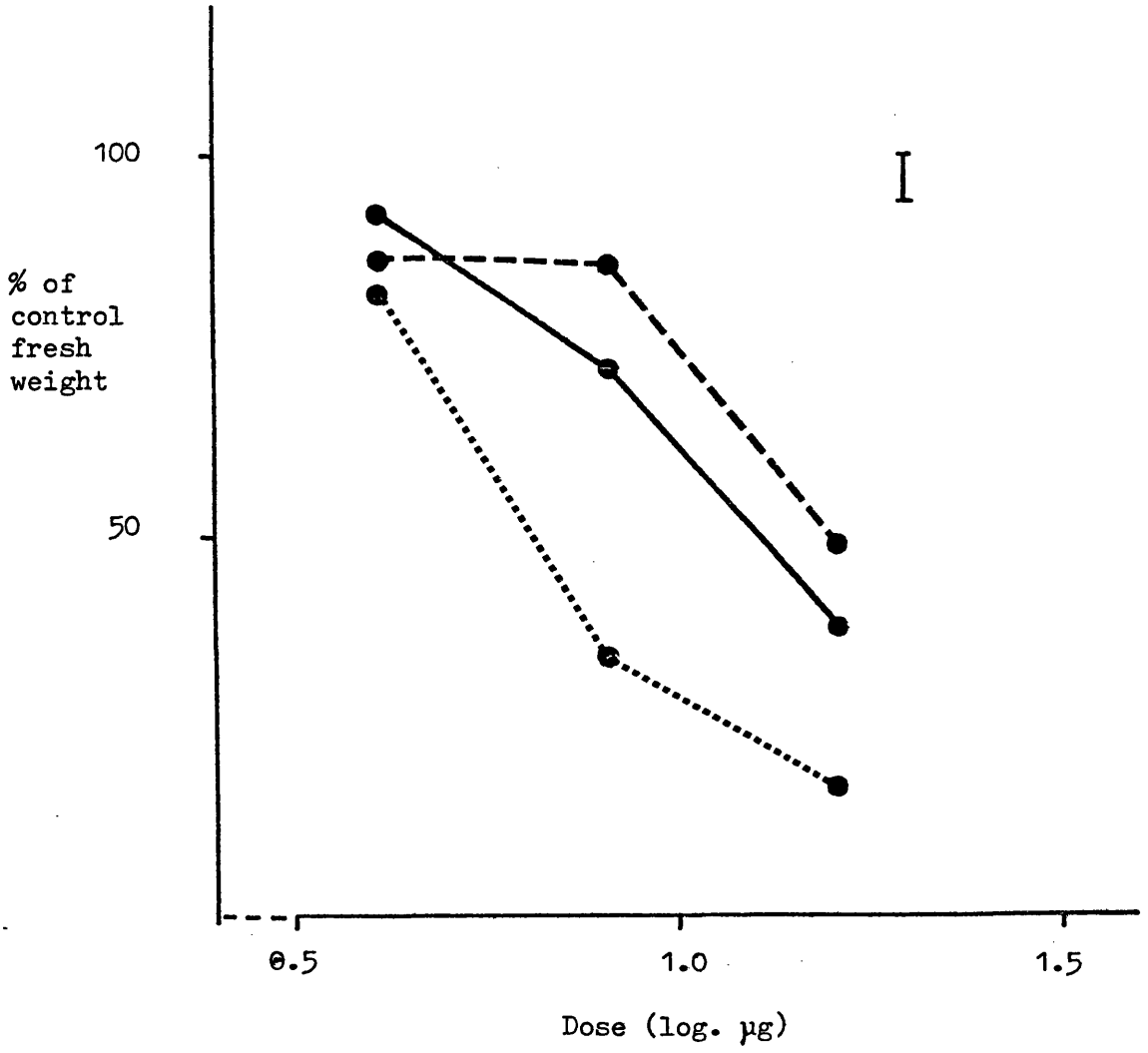


Figure 4.38: The response of radish (2 leaves) to glyphosate applied at three doses to three positions.

Positions: Foliar leaf laminae .....

Foliar leaf veins - - -

Cotyledons ———

is possible that glyphosate relies more on application to regions of phloem-loading or that the surface layers of the midrib are less permeable to glyphosate than the leaf lamina even though the reverse may be true with MCPA and paraquat.

4.3.6 Experiments with glyphosate on wild oat

4.3.6.1 The dose response. In a preliminary experiment three-leaf wild oats were treated using a solution containing 72 $g.l^{-1}$ glyphosate with doses in the range 2.05 - 32.7 µg as 2-32 drops of 300 µm diameter. All the treated plants were killed at these doses so a second experiment was conducted; because a suitable range of lower numbers of drops could not be applied a less concentrated solution was used (9.0 $g.l^{-1}$). Doses of 0.13 - 2.05 µg were applied to the second leaf of wild oats with three leaves and a mean fresh weight of 0.37 grams. The treatments comprised 1-16 drops of 300 µm diameter. Fresh weight was assessed after 19 days.

As with radish there were no visible symptoms of glyphosate for about one week. New growth which subsequently developed was chlorotic and at the lower doses there was some proliferation of stunted and chlorotic tillers. The results (Figure 4.39) showed a highly significant dose response ($p = 0.01$). Although it is not obvious from Figure 4.39, the highest dose represented about the maximum possible effect since most plants died at this dose.

4.3.6.2 The effect of concentration. Two experiments were conducted on the effect of concentration of glyphosate on wild oat. In the first experiment drops of 300 µm were used, and in order to apply what was thought to be the right dose range from the experiment described in

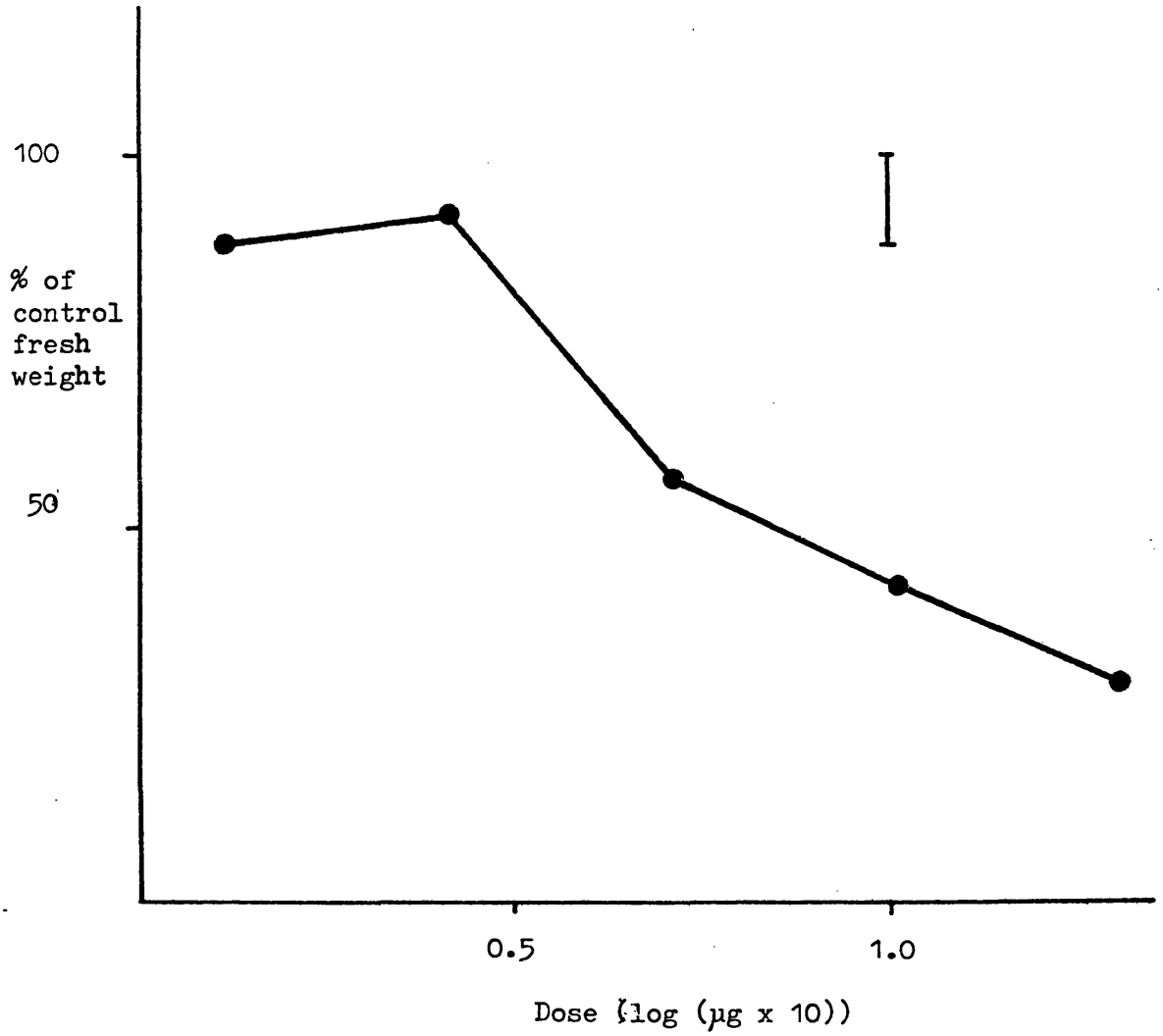


Figure 4.39: The response of wild oat (three leaves) to a range of doses of glyphosate, applied as 300 µm drops using a concentration of 9.0 gl^{-1} a.e.

Section 4.1.6.1 concentrations of 9, 18 and 36 $g.l^{-1}$ were used. Doses were 0.5, 1.0 and 2.0 µg applied to leaf 2 of wild oats with $2\frac{1}{2}$ leaves and a mean fresh weight of 0.23 grams. Fresh weight was assessed after 20 days.

The results of this experiment (Figure 4.40) showed a highly significant dose response ($p<0.001$) but no significant effect of concentration. This result contrasts somewhat with that obtained with radish (Section 4.3.5.2) although a lower range of concentrations were applied in this experiment.

To examine the higher concentrations on wild oat a second experiment was conducted using concentrations of 36, 72 and 144 $g.l^{-1}$. To ensure that sub-lethal doses could still be examined the drop size used had to be reduced, and 200 µm drops were used. Doses of 0.61, 1.21 and 2.41 µg were applied which still only required 1, 2 and 4 drops at the most concentrated solution. Plants had 3 leaves plus 2 small tillers and a mean fresh weight of 0.36 grams, the glyphosate treatments being applied to the second leaf. As with the previous experiment fresh weight was assessed after 20 days.

The general level of glyphosate effect was so high in this experiment that no differences between concentrations at the highest two doses were shown, despite a highly significant dose response ($p<0.001$) as shown in Figure 4.41. However at the lowest dose the least concentrated solution was less effective than the more concentrated solutions. This result was correlated with the appearance of the plants since those with the lowest concentration were recovering from early herbicide symptoms and noticeably more healthy than plants treated with the higher concentrations. Thus there is a suggestion in this experiment of the same concentration effect seen with radish where

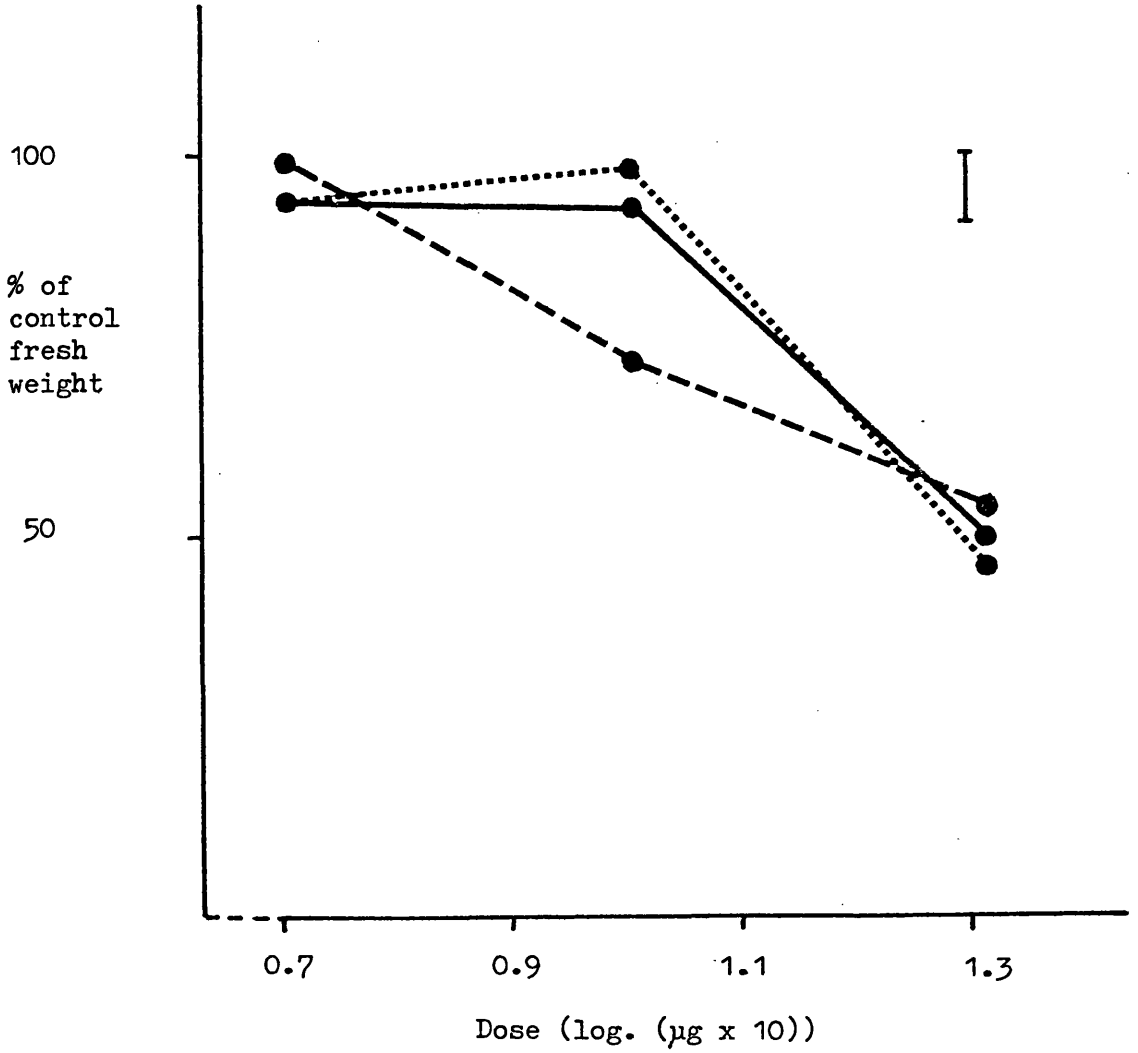


Figure 4.40: The response of wild oat ($2\frac{1}{2}$ leaves) to glyphosate at three doses and three concentrations, using 300 µm drops.

Concentrations: 9 gl^{-1} .....

18 " – – –

36 " ——

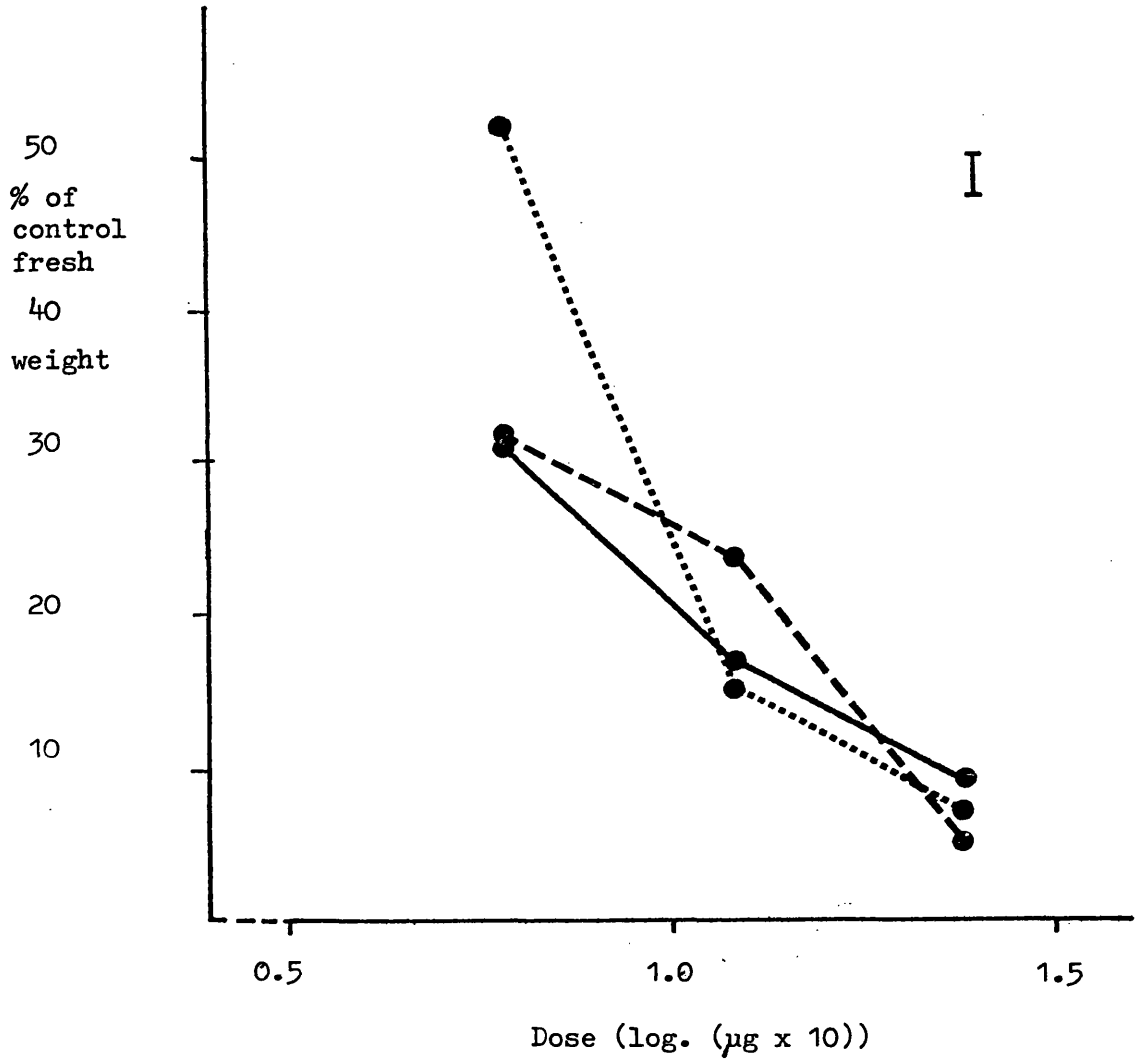


Figure 4.41: The response of wild oat (3 leaves plus 2 tillers) to glyphosate at three doses and three concentrations using 200 µm drops.

Concentrations: 36 gl^{-1} .....

72 " - - -

144 " ——

higher concentrations are more effective, although the effect is less clear, and it may be that enhanced glyphosate activity only occurs at concentrations greater than about 36 $g.l^{-1}$.

4.3.6.3 The effect of position of deposit. Plants with three leaves and one small tiller were treated with doses of 0.30, 0.61, 1.21 and 2.42 μg as 1, 2, 4 and 8 drops of 300 μm diameter, using a concentration of 72 $g.l^{-1}$ of glyphosate. The treatments were applied to leaves 1, 2 or 3, in each case between 20 and 60 mm from the ligule. Fresh weight was assessed after 22 days.

A highly significant dose response was observed ($p<0.001$). (Fig. 4.42). Although treatment to the first leaf tended to cause less reduction in fresh weight at three doses than treatment to the other leaves, there were no significant differences due to position. This is contrary to the results of similar experiments with difenzoquat and paraquat (Sections 4.3.2.4 and 4.3.4.3) where the first leaf was considerably less effective as a site of treatment. This result with glyphosate suggests that there is no major reduction in the efficiency of translocation from the older leaf although it does not preclude the possibility that leaf 1 may be more susceptible to localised damage, which may subsequently interfere with difenzoquat and paraquat movement, since glyphosate does not apparently cause such damage. These results are very similar to those observed with glyphosate on three-leaf couch plants (Coupland, Taylor and Caseley, 1978) who suspected that there might be some difference between the translocation system of couch and wild oat since several wild oat herbicides were less effective on older leaves. This experiment would suggest that there is no difference in the translocation systems, but that the observed differences between glyphosate and wild oat herbicides are connected with the herbicide mode of action.

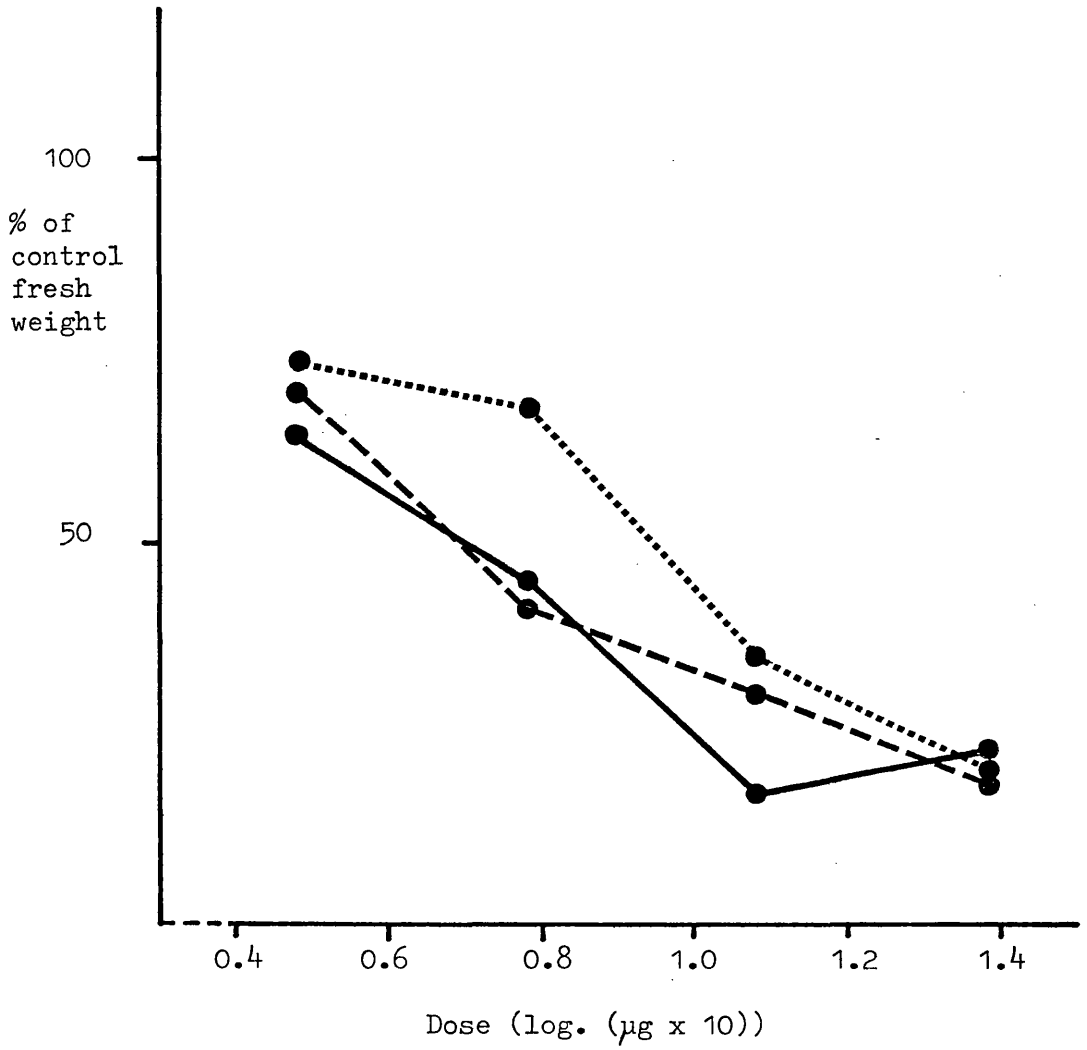


Figure 4.42: The response of wild oat (3 leaves plus 1 tiller) to glyphosate at four doses applied to three positions.

Positions: Leaf 1 ·····

Leaf 2 – – –

Leaf 3 ———

4.4 The uptake and movement of difenzoquat

It is possible that a major effect of changes in form of deposit might be to alter the rate or extent of uptake and movement of herbicide. It was suggested in Section 4.3.2 that this may be the cause of differences between some treatments with difenzoquat, possibly due to the severity of observed physical damage at the site of treatment. Therefore experiments were carried out to study the uptake and movement of ^{14}C-labelled difenzoquat by the methods described in Section 3.7.

4.4.1 The effect of method of application of labelled difenzoquat

In all previously reported experiments on uptake and movement of radio-labelled herbicides, the herbicide has been applied in relatively large volumes, typically either as large drops produced by microsyringes or in wells attached to the plant. The development of the single drop applicator described in Section 3.3.1 made it possible to apply labelled herbicide in actual spray drop sizes. This was considered essential for the study of uptake and movement in relation to controlled drop application because the use of large drops would lead to considerable over-dosing at the site of treatment due to the high concentration of herbicide necessary. Application by microsyringe may therefore result in local toxic responses of a level not normally encountered from sprayed deposits, although it is acknowledged that this may not be so much of a problem with previously reported work relating to conventional applications at much lower herbicide concentrations.

With these points in mind an experiment was carried out to compare the application of labelled difenzoquat in a high concentration of 50 $g.l^{-1}$ as 32 drops of 300 μm diameter (0.0142 μl) using the single drop applicator, with a lower concentration of 5 $g.l^{-1}$ as 9 drops of

985 μm diameter (0.5 μl) applied from a microsyringe. As a further treatment factor the applications were either restricted to 20 mm of leaf (50-70 mm from the ligule of leaf 2) or dispersed over 60 mm of leaf (30-90 mm from the ligule). The plants had 3 leaves at treatment and were harvested after 24 and 72 hours from the treatment time, these being times that should permit entry and movement of measurable quantities of difenzoquat (Sharma et al, 1976).

The amount of radioactivity was determined in a washoff, the treated leaf and the rest of the plant (including both roots and shoot) at each harvest time, the samples being prepared for scintillation counting as described in Section 3.7. It has been previously stated that difenzoquat is not significantly metabolised in plant tissues within the time scale of this and other experiments reported here, so that the amount of radioactivity detected can be taken as equivalent to the amount of herbicide present in the tissue (Sharma et al, 1976).

There were no visible symptoms of herbicide injury after 24 hours. By 72 hours the beginnings of scorch were evident on the treatments confined to 20 mm of leaf, with identation of leaf tissue and some loss of pigmentation. These symptoms were more severe with the 985 μm drops than with the 300 μm drops.

Analysis of the results showed a highly significant effect of sampling time for all treatments indicating that entry and movement occurred over the sampling period studied. Table 4.4.1 shows the percentage of radioactivity recovered in each sample along with transformed data upon which analysis was performed. Between 41 and 80% of the applied herbicide was recovered in the washoff sample over the time period studied, whilst the amounts which entered and were subsequently

Table 4.4.1 Difenzoquat uptake and movement from various methods of application: the recovery of ^{14}C-labelled difenzoquat from wild oat plants as a percentage of the total recovery.

Figures in brackets are data following angular transformation as used in statistical analysis.

Method of application	Length of leaf treated, mm	Sample time (hours)	% of total label recovered + (data following angular transformation) Washoff	Treated leaf	Rest of plant
32 x 300 µm drops	20	24	80 (64)	20 (26)	0.03 (0.7)
		72	41 (40)	55 (48)	4.03 (11.2)
	60	24	84 (67)	16 (24)	0.07 (1.2)
		72	51 (45)	46 (43)	3.78 (10.8)
9 x 985 µm drops	20	24	68 (55)	32 (35)	0.02 (0.4)
		72	58 (50)	42 (40)	0.43 (3.5)
	60	24	68 (55)	33 (35)	0.04 (0.7)
		72	41 (40)	58 (50)	0.74 (4.6)
	STANDARD ERROR		(2.4)	(2.4)	(1.1)

translocated toother plant parts were between 0.02 and 4.03% of that applied, indicating the small amount of difenzoquat that is responsible for activity at the shoot apex. Results obtained by Caseley and Coupland (1980) suggest that the amount of herbicide reaching the apical region of the shoot is of the order of 20% of that found in the plant other than the treated leaf. Thus the quantity of the applied active ingredient which ultimately reaches the shoot apex may be very small; the results of this experiment would suggest a figure of 0.08 - 0.8% of the total applied reaching the apex by 72 hours.

There were significant differences between the 985 μm and 300 μm drop treatments for samples of both treated leaf ($P<0.001$) and rest of plant ($p = 0.01$). Although no significant main effect of method of application was apparent with the washoff sample, this was probably due to a significant interaction between this factor and time. Comparing the washoff and treated leaf samples as inverse measures of herbicide entry it appears that entry was more rapid with the 985 μm drops up to 24 hours. By 72 hours more herbicide had entered with the 300 μm drops in the case of the treatments restricted to 20 mm of leaf, and with the more dispersed treatments the differences were no longer significant. Also at the 72 hour sample time significantly more herbicide was translocated out of the treated leaf with the 300 μm drops than with the 985 μm drops, as judged by the rest of plant samples. No significant differences in translocation were apparent by 24 hours, largely due to the very low quantities detected at this sample time. There were no significant differences between the restricted and dispersed treatments with respect to translocation at either sample time.

In summary, it appears that the use of 985 μm drops caused greater initial entry of difenzoquat than 300 μm drops of a more

concentrated solution, but that subsequent movement of the herbicide out of the treated leaf was reduced with the larger drops. Factors which varied between these treatments included the size and number of drops, the initial herbicide concentration and that of the dried deposit, the relative amounts of herbicide to surfactant and the rate of drying of the drops. Therefore it is not possible to identify the factor responsible for the differences in uptake and movement, although it seems probable that these differences were associated with local injury due to the greater concentration of the herbicide deposit with the larger drops. The experiment also demonstrated that the manner in which the herbicide is applied, that is the drop size and concentration can affect the uptake and movement.

4.4.2 The effect of difenzoquat concentration

In Section 4.3.2.2 it was shown that applications of different concentrations of difenzoquat in uniform drops of 300 μm caused differing herbicide performance, with the least concentrated treatments being the most effective. To study the effects of such treatments on difenzoquat uptake and movement the highest and lowest concentrations used in the biological performance experiments, namely 25 and 200 $g.l^{-1}$, were applied using solutions containing labelled difenzoquat. 300 μm drops were used to apply a single dose of 22.7 μg of difenzoquat, using 8 or 64 drops of the 200 and 25 $g.l^{-1}$ solutions respectively. Again radioactivity was determined in washoff, treated leaf and rest of plant samples. Sampling times were 24 and 72 hours plus a third time of 10 days after treatment to study the longer term movement of difenzoquat after penetration was complete and necrosis had fully developed.

Observations of symptoms at the site of treatment were recorded for each sample time. At the 24 hour sample time there were no signs of

necrosis with either treatment, but some indentation of the leaf surface was apparent with some drops of the 200 $g.l^{-1}$ solution. At 72 hours most drops with this concentration had caused necrotic lesions which extended to approximately the circumference of the original drop stain. In contrast very few drops of the 25 $g.l^{-1}$ solution had caused necrosis at this time. By the 10 day sample time severe necrotic lesions had formed around each drop with the 200 $g.l^{-1}$ solution, each lesion being about 3 mm in diameter, with tissue around these lesions remaining relatively undamaged. With the 25 $g.l^{-1}$ solution some variability in degree of necrosis occurred, with a range of symptoms between small lesions around each drop to a general necrosis of the treated area.

The results of the radioactivity determinations are shown in Table 4.4.2. With the 200 $g.l^{-1}$ treatment the amount of radioactivity in the wash-off sample was 93% of that applied after 24 hours falling to 26% by 72 hours and 0.2% by 10 days. With 25 $g.l^{-1}$ entry was more rapid with less radioactivity in the 24 and 72 hour wash-off samples. These differences were reflected in the treated leaf samples showing that initial penetration was greater with the more dilute solution, although entry was almost complete with both concentrations by 10 days. Movement of difenzoquat out of the treated leaf was also similar for both concentrations by 10 days after treatment, with about 11% of the applied amount recovered from outside the treated leaf at this time. However, there was greater movement of difenzoquat from the 25 $g.l^{-1}$ concentration at the 72 hour sample time. At 24 hours after treatment there was no significant difference between translocation with the two concentrations, although the amounts translocated by this time were low. It therefore appears that entry and movement of difenzoquat over the 1-3 day period after application to wild oat leaves are greater with a more dilute treatment solution. The relationship between these results and the

Table 4.4.2 Difenzoquat uptake and movement from application of two concentrations: the recovery of ^{14}C-labelled difenzoquat from wild oat plants as a percentage of the total recovery.
Figures in brackets are data following angular transformation as used in statistical analysis.

Concentration $g.l^{-1}$	Sample time	Washoff	Treated leaf	Rest of plant
200	24 hours	93 (76)	7.2 (14)	0.12 (1.3)
	72 hours	26 (28)	73 (60)	1.26 (5.9)
	10 days	0.2 (2.3)	96 (79)	3.36 (10.9)
25	24 hours	69 (56)	31 (34)	0.03 (0.6)
	72 hours	6.2 (14)	91 (73)	2.51 (9.1)
	10 days	0.1 (1.5)	96 (79)	3.87 (11.3)
STANDARD ERROR		(2.5)	(2.3)	(0.6)

observations of necrosis at the treated site suggest that the critical period over which differences in entry and movement occur is around or just before the time at which visible necrotic symptoms appear.

It would seem from the results in Section 4.3.2.2 that this effect is important in determining the biological response. Possibly, as the plant continues to grow, the dilution factor of the translocated herbicide increases, so that although the amount of difenzoquat eventually moving out of the treated area was the same for both treatments by 10 days after application, that which moved at the later stages of this sample period were of lesser importance in determining the biological response.

4.4.3 The effect of leaf age on difenzoquat entry and movement

It was shown in Section 4.3.2.4 that difenzoquat was less effective when applied to older leaves of wild oat. As with the effect of herbicide concentration there was a suggestion that this might be due to a reduction in translocation through injury to leaf tissue at the site of treatment. Therefore an experiment was designed to study entry of difenzoquat into, and movement from leaves of different ages.

A solution of difenzoquat containing 50 $g.l^{-1}$ including ^{14}C-labelled herbicide was applied as 32 drops of 300 μm diameter to give a dose of 22.7 μg per plant. This dose was applied to leaves 1, 2 or 3 of three leaf wild oat plants, in each case treating a portion of the leaf 30-90 mm from the ligule. The same samples as in previous experiments were assayed at 24 and 72 hour sampling times after application.

As before there was a highly significant difference between sampling times with all three samples. With washoff and treated leaf samples there was no effect of leaf age, but an interaction between

leaf age and time of sampling. The results (Table 4.4.3) show that this interaction is due to a change in the pattern of entry into the leaves over the sample period. After 24 hours there was a greater entry of difenzoquat into leaf 1 than leaf 3, with the value for leaf 2 falling between these. After 72 hours this trend was reversed with the greatest penetration into the youngest leaf, leaf 3.

With the samples representing movement out of the treated leaf to the rest of the plant, there were no significant differences at the 24 hour sample time. However by 72 hours there was greater movement from the treatments applied to leaf 3 than from leaves 1 or 2.

Thus the biological response observed in the previously described experiments correlated with the degree of movement of difenzoquat determined here. It is possible that the greater capacity of leaf 3 to translocate difenzoquat was responsible for the observed greater uptake after 72 hours by increasing the diffusion gradient into the leaf tissue. Conversely it may be that rapid initial entry into leaf 1 resulted in greater local injury which then restricted later uptake and movement. Either of these explanations would be consistent with the observation that necrosis was most severe when difenzoquat was applied to older leaves (Section 4.3.2.4).

Table 4.4.3 Difenzoquat uptake and movements from leaves of different ages: the recovery of ^{14}C-labelled difenzoquat from wild oat plants as a percentage of the total recovery.
Figures in brackets are data following angular transformation as used in statistical analysis.

Leaf	Sample time (hours)	Washoff	Treated leaf	Rest of plant
1	24	69 (57)	31 (33)	0.08 (1.3)
	72	48 (44)	50 (45)	1.26 (6.0)
2	24	75 (61)	25 (29)	0.06 (0.9)
	72	40 (39)	58 (50)	1.96 (8.0)
3	24	78 (63)	22 (27)	0.25 (2.7)
	72	30 (33)	66 (55)	3.46 (10.7)
	STANDARD ERROR	(2.8)	(2.8)	(0.7)

4.5 Observations on the sites of entry of fluorescent dyes

In the studies on the effect of form of deposit on herbicide performance (Section 4.3) it was observed that the necrotic effects of herbicide drops were variable. For example with some treatments of difenzoquat some drops caused severe necrosis while others caused none. Because the degree of necrosis generally increases with leaf age, it is possible that areas of severe necrosis might indicate that the drop had been applied to a site already damaged, causing locally increased herbicide entry. Such damage could be caused by, for example, wind giving rise to abrasion by sand particles or other leaves, or alternatively as part of the natural ageing process. To test these possibilities an attempt was made to identify the sites of entry of externally applied chemicals.

One technique which has been used to indicate the entry of water and solutions into plant material involves tracing with fluorescent dyes. This technique has been used to study the entry of solutions into stomatal pores (Dybing and Currier, 1959 and 1961) and the role of trichomes in the regulation of water passage into and out of leaves (Butterfass, 1956). The fluorescent dye used in much of this work was 3-hydroxy - 5,8,10 - pyrenetrisulphonate (PTS), a known apoplastic chemical (Peterson and Edgington, 1976). Butterfass also used a number of other dyes in his work.

In the present studies a method based on that of Dybing and Currier (1959 and 1961) was used to study the sites of entry of a range of fluorescent dyes into the leaves of several plant species. This technique, described in Section 3.8, permitted only qualitative observation and photographic recording of the sites of entry.

4.5.1 The entry of fluorescein into leaves of wild oat (Avena fatua)

Solutions containing 1.0 $g.l^{-1}$ fluorescein sodium salt and 0.01, 0.1 or 1.0% v/v Agral were applied to wild oat leaves as large drops of about 2 µl volume. Surface deposit was removed by washing after 15 minutes and the sites of dye remaining in the tissue observed using blue excitation light. Greatest entry of dye, as judged by the intensity and extent of yellow-green fluorescent light, occurred with 0.1 and 1.0% Agral solutions. The location of the dye seemed to be predominantly the stomatal guard and accessory cells. Dye was apparently concentrated in cell walls particularly the thickened central walls of the guard cells, but evidence of entry into the cytoplasmic components of the guard and accessory cells was provided by the obvious concentration of the fluorescence in the nuclei and other sub-cellular organelles, including the chloroplasts. These general features of fluorescein location are demonstrated in Plate 4.3a. A further aspect of the distribution of the dye was that the degree of entry varied considerably from one place to another. For example, a brightly fluorescent stoma was frequently adjacent to a non-fluorescent one (Plate 4.3b). There was no obvious reason for such differences, which may have been due to differences in surface structure or permeability of the plasmalemma, or alternatively in contact of the spray liquid with the cell surface. A high degree of entry also occurred around some trichomes (Plate 4.4a), and at leaf edges (Plate 4.4b). These examples may be related to mechanical damage of the leaf surface, since both features (trichomes and leaf edges) would be more prone to such damage when wind causes leaves to rub together.

The degree of entry of fluorescein varied according to the time during which the dye solution was allowed to remain on the leaf. Using

Plate 4.3 Distribution of fluorescein in wild oat leaves;

a. showing location of dye in stomata.(X200)

b. a brightly fluorescent stoma adjacent to a non-fluorescent one.(X200)

Plate 4.4 Regions of high entry of fluorescein on wild oat leaves;

a. around a trichome. (X200)

b. at a leaf margin.(X100)

2 µl drops of a solution containing 0.5% Agral and 1.0 $g.l^{-1}$ fluorescein, applied to the third leaf of 4-leaf plants, a visual estimate of the proportion of stomata containing dye was made. After 5 minutes approximately 60% of the stomata fluoresced whilst by 15 minutes the proportion had risen to about 80%, this rising further to 95% by 30 minutes and 100% by 60 minutes. Plate 4.5a shows an example of a leaf exposed to dye solution for 30 minutes, and demonstrates also that the intensity of fluorescence varied considerably from cell to cell.

Comparing different leaves on the wild oat plants it was observed that penetration of fluorescein from a 1.0 $g.l^{-1}$ solution containing 0.5% Agral in 2 µl drops was generally more rapid on older leaves, for example after 5 minutes many more cells on leaf 1 were fluorescent than on leaf 3 under comparable conditions (Plate 4.5b,c). Similar results were obtained when 300 µm drops of the same solution were applied. Here, possibly due to the faster drying of the smaller drops, it took longer for the fluorescence to reach the same level in the plant cells than with 2 µl drops. The amount of dye which had entered after 1 hour into leaves 1, 2 and 3 of a four-leaf wild oat plant can be seen in Plate 4.6a, b and c respectively. There appears to be more dye present in the walls of the epidermal cells other than the stomata with the older leaves which may indicate that the cuticle as a whole becomes less resistant to the entry of solutions as leaves age.

4.5.2 The entry of PTS into leaves

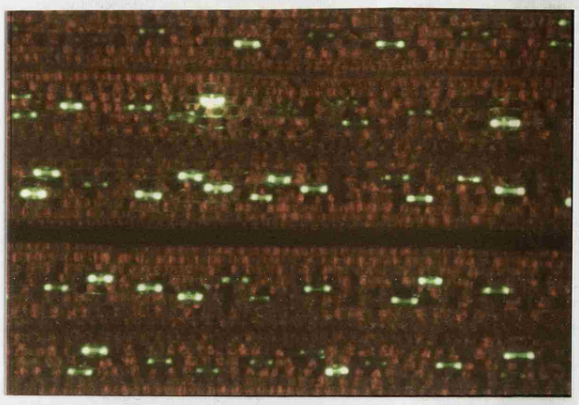
As was mentioned above the dye PTS is known to be almost entirely restricted to the apoplast of plant tissues. Therefore the entry of this dye was compared with that of fluorescein, since the observations with fluorescein described in the preceding section were

Plate 4.5 Location of fluorescein in wild oat leaves:

a. a leaf treated with dye solution for 30 minutes. (X60)

b. entry after a 5 minute treatment on leaf 1. (X60)

c. entry after a 5 minute treatment on leaf 3. (X60)



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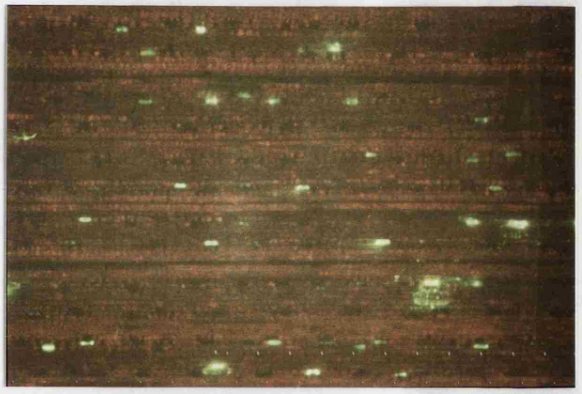

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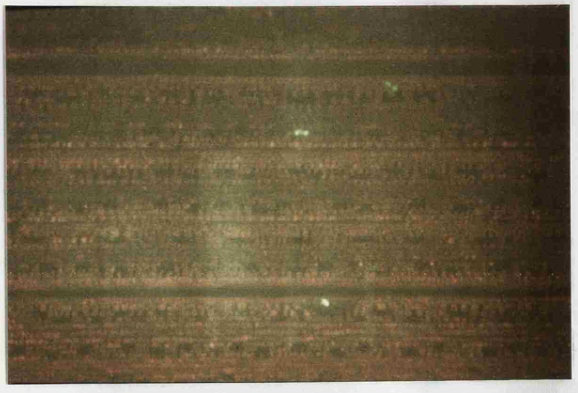


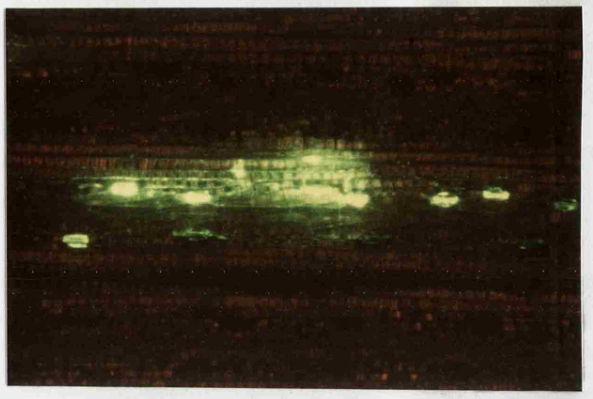
c

Plate 4.6 Entry of fluorescein in wild oat leaves 1 hour after treatment with 300 µm drops of solution;

a. leaf 1 (X60)

b. leaf 2 (X60)

c. leaf 3 (X60).



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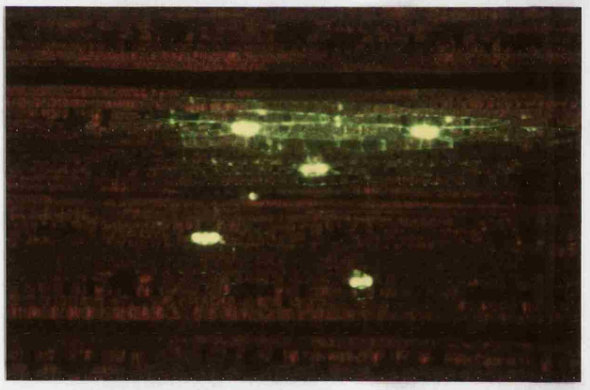

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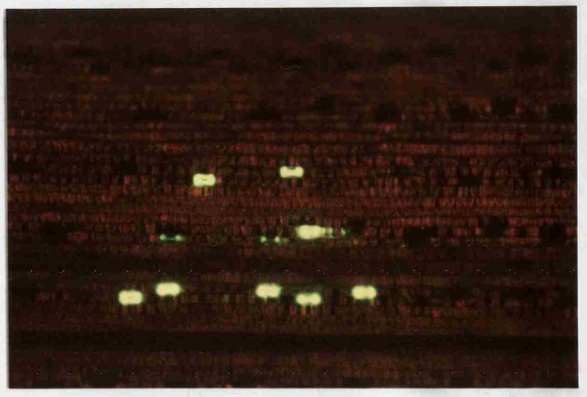


c

in marked contrast to those with PTS on leaves of Zebrina pendula reported by Dybing and Currier (1959, 1961).

Firstly the method of Dybing and Currier was followed as closely as possible, applying to the underside of Zebrina leaves a solution containing 1.0 $g.l^{-1}$ of PTS and 0.1% KO2 (sodium di-iso-octyl sulphosuccinate), an anionic surfactant similar to that used by these authors. The underside was used because the upper surface is astomatous with this species. The entry of dye was found to be as described by Dybing and Currier. Patches of fluorescence around some stomata extended several cells away from the stomatal pore indicating that solution had entered the sub-stomatal cavities (which were brightest in fluorescence) and began to diffuse through intercellular spaces and cell walls (Plate 4.7a). Having observed this effect the work was repeated using a solution of 1.0 $g.l^{-1}$ fluorescein instead of PTS. As expected the patches of cells around stomata, indicating stomatal pore penetrations, were seen with fluorescein as with PTS; but in addition with fluorescein many stomatal guard cells showed fluorescence whilst there was no evidence of penetration through the pores of such stomata (Plate 4.7b), this agreeing with the observations on wild oats in Section 4.5.1. In order to verify these observations it was found possible to identify the sites of entry of both fluorescein and PTS from a solution containing both dyes at 1.0 $g.l^{-1}$. This relied on the different wavelengths of excitation and emission of the two dyes. PTS is excited by light of ultra-violet to violet wavelengths (peak 469 nm) and fluoresces blue-green (peak 516 nm) whilst fluorescein is excited by blue light (peak 493 nm) and fluoresces green (peak 514 nm). Using suitable filter combinations it was possible to confine observations to either dye from a mixed application with virtually no interference by the other dye. These observations confirmed that whilst fluorescein penetrated those sites which PTS

Plate 4.7 Location of dyes applied to the underside of *Zebrina pendula* leaves;

a. PTS (X200)

b. fluorescein (X200).

entered, many more sites were penetrated by fluorescein alone, in particular many guard cells and accessory cells (Plates 4.8 (Zebrina) and 4.9 (wild oat).

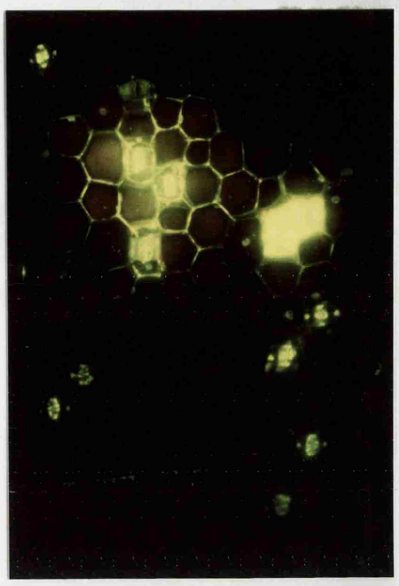
Using a solution of 1.0 $g.l^{-1}$ PTS and 0.5% Agral the entry of this dye on wild oat leaves was studied, again using 2 µl drops. It was found that penetration of PTS was generally poor into these leaves. However, a small proportion of stomata were clearly entered via the stomatal pore. If samples were observed after about 5 minutes small drops of dye solution could be seen immediately below the stomata (Plate 4.10a). If left for 10-15 minutes these drops had dispersed to a greater area, apparently concentrated in the cell walls (Plate 4.10b). The occurrence of these sites of stomatal pore entry was however low, being estimated at between 1 and 5% of stomata within the area covered by the drop.

4.5.3 The entry of other fluorescent dyes into wild oat leaves

A range of dyes were applied in 2 µl drops to wild oat leaves in order to examine the effect of varying chemical structure on the pattern of uptake. Each dye was used as an aqueous solution containing 5 $g.l^{-1}$ of the dye and 0.1% Agral surfactant. The leaves were treated for 10-15 minutes before washing off the surface deposit, during which time the drops did not dry. The dyes used were eosin, erythrosin, rose bengale, rhodamine B, coriophosphine, acridine orange, thioflavine T and primuline and their structures are shown in Figure 4.43. These dyes were chosen since they represent four groups of chemically related compounds. Eosin, erythrosin and rose bengale are fluorones (or hydroxyxanthenes) and thus related to fluorescein, but having various substitutions. These are all sodium salts, and therefore anionic dyes. Rhodamine B is a fluorene (or aminoxanthene), which are xanthene dyes

Plate 4.8 A comparison of the distribution of PTS and fluorescein in leaves of Zebrina pendula (abaxial surface) following application of a mixed solution: a and c both show fluorescein location; b and d show PTS location. a and b are the same portion of leaf as are c and d.

(a, b X45; c, d X120)



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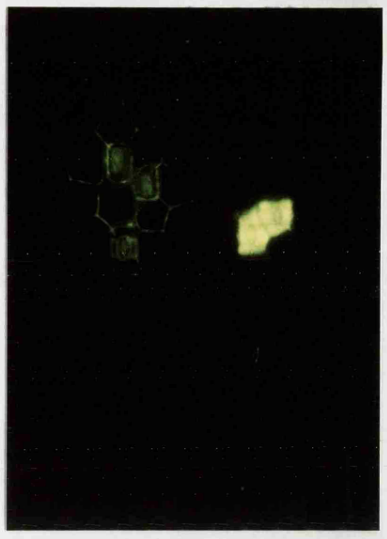

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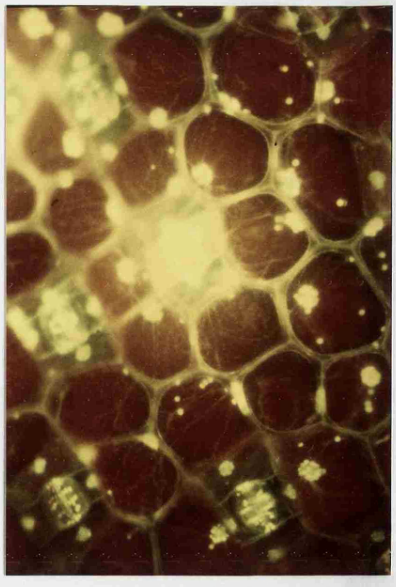

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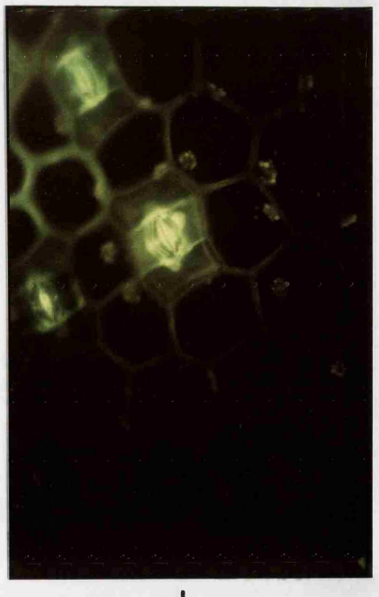
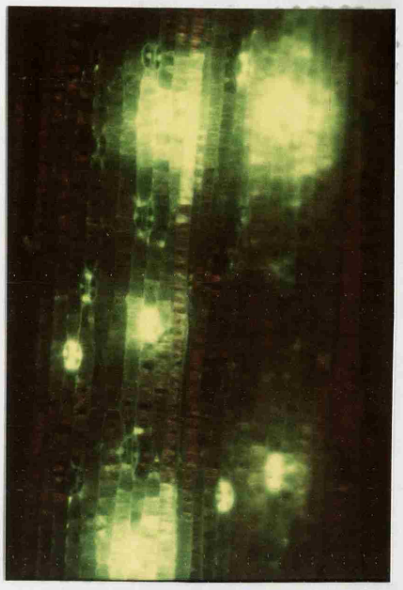


d

Plate 4.9 A comparison of the distribution of PTS and fluorescein in leaves of wild oat following application of a mixed solution: a and c show fluorescein location, b and d show PTS location. a and b are photographs of an area showing substantial entry of PTS, whilst c and d show an area where little PTS entry occurred.

(all X60)



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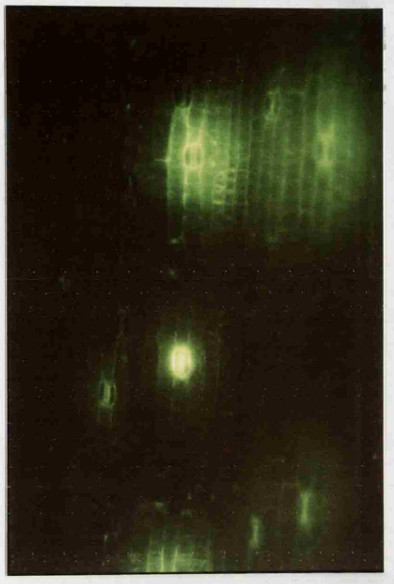

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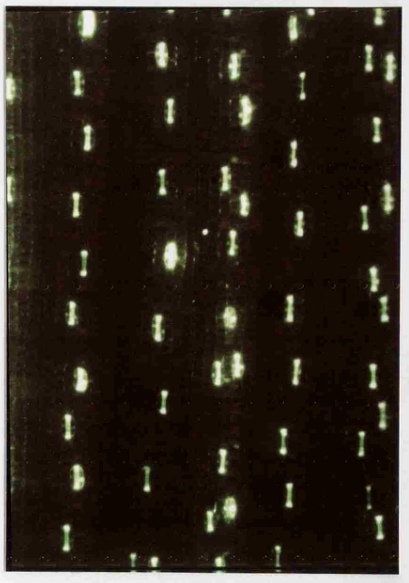

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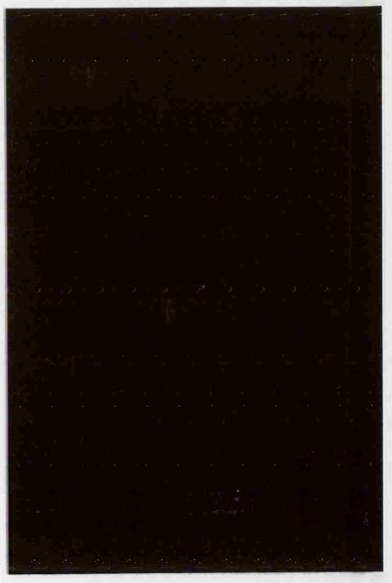


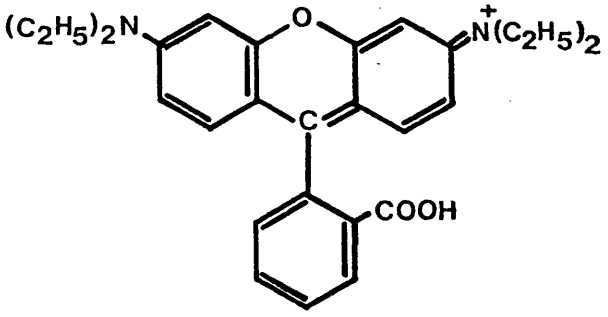
d

Plate 4.10 The entry of PTS through the stomatal pores of wild oat leaves.

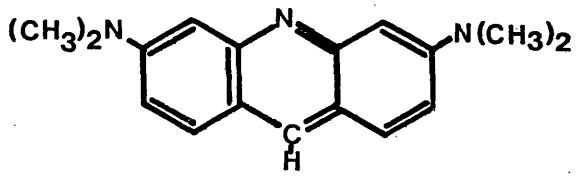
a. droplets of dye solution in the sub-stomatal cavity just after entry. (X200)

b. a later stage showing spread of the dye through cell walls and inter-cellular spaces. (X200)

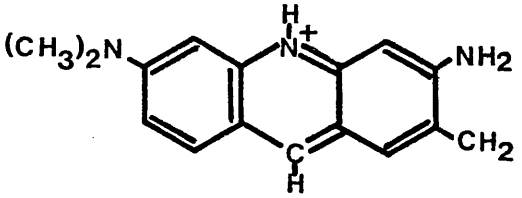
Rhodamine B



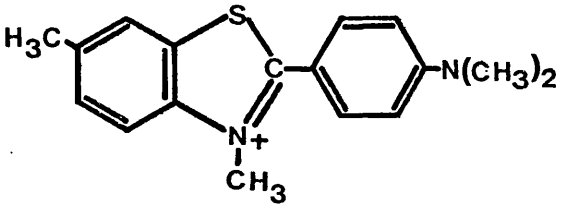
Acridine orange



Coriophosphine



Thioflavine T



Primuline

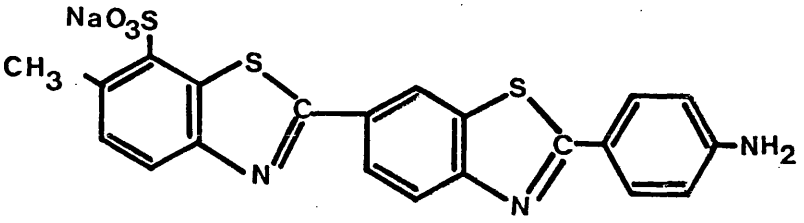
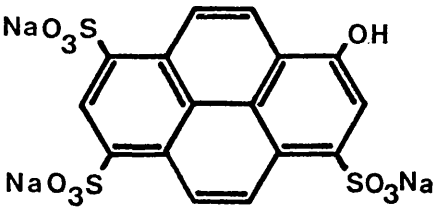


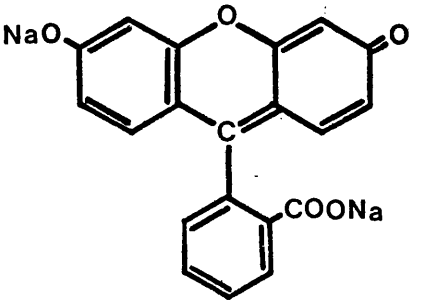
Figure 4.43

Structures of the fluorescent dyes used in these studies

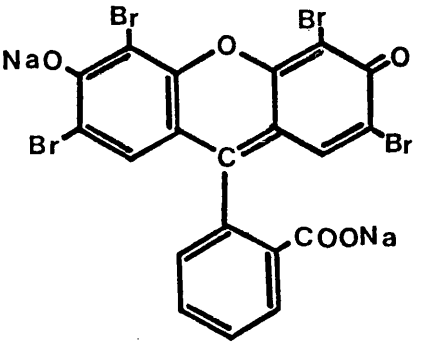
PTS



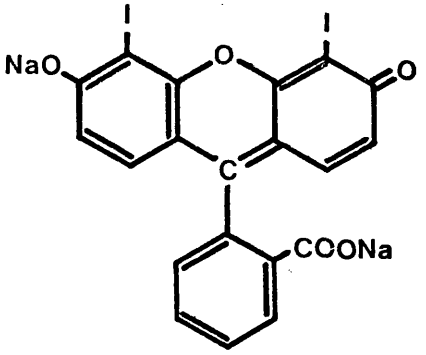
Fluorescein



Eosin



E rythrosin



Rose bengale

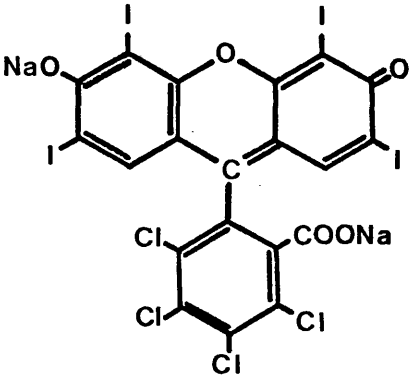


Figure 4.43

Continued

like the fluorones, a major difference being that rhodamine B is a cationic dye and chloride salt. Coriophosphine and acridine orange are both cationic dyes (again chloride salts) and are representatives of the acridine group of dyes being based on acridine, which is chemically similar to xanthene. The final two dyes, primuline and thioflavine T are thiazoles, primuline being a sodium salt and anionic dye, whilst thioflavine T is a chloride salt and cationic dye.

In observing the distribution of absorbed dye the optimum visibility had to be determined first with the four available filter combinations, which permit four different wavelengths of excitation light (ultra-violet, violet, blue and green).

Eosin gave yellow fluorescence under blue excitation light. The distribution pattern was very similar to that of fluorescein, which may be due to its structural similarity to that compound, being a tetra-bromo derivative. As with fluorescein most stomatal guard cells were stained with the dye localized in intracellular organelles such as chloroplasts and nuclei (Plate 4.11a).

Erythrosin was much less readily visible than fluorescein and eosin. This may be due to the two iodine atoms restricting movement across membranes, or simply to this dye being less brightly fluorescent. Despite the low intensity of its fluorescence it was possible to see that the distribution of the dye was very similar to that of fluor-escein (Plate 4.11b).

The final dye of this group, rose bengale, again showed fairly low intensity fluorescence, and visibility was further hindered because the fluorescent light was of a red colour very similar to the back-ground fluorescence of the plant pigments. However it was still possible

Plate 4.11 Location of fluorescent dyes in wild oat leaves:

a. Eosin (X200)

b. Erythrosin (X200).

to see that the dye was concentrated in the guard and accessory cells of the stomata, with some entry into the adjacent epidermal cells even though photographic recording of this dye proved unsuccessful. Thus all the dyes in the fluorone group seem to be localised in the stomata at least during the initial 15 minutes of entry, and penetration into the cells themselves clearly occurs, although possibly less so with the more halogenated derivatives.

Rhodamine B, like rose bengale, produces a red fluorescence, although the greater brightness with rhodamine B facilitates observation with this dye. Again dye was particularly associated with guard and accessory cells of stomata, with approximately 10-20% of these brightly fluorescent after 15 minutes treatment. The fluorescence here was not confined or localised to identifyable sites within the cells but appeared general. This led to the conclusion that this dye is either generally dispersed throughout the cells, or possibly confined to the cell walls, which would give a similar appearance since these completely surround the cell contents. In addition there were a number of patches of brightly fluorescent epidermal cells around stomata suggesting either stomatal pore entry or local differences in cell permeability to entry of the dye (Plate 4.12a).

Coriophosphine entered small patches of epidermal cells, usually associated with trichomes or stomata although some were isolated from these features. As with rhodamine B this dye was not localized in intracellular organelles suggesting complete entry or confinement to walls. Acridine orange, very similar to coriophosphine in structure, showed virtually identical distribution characteristics; with both these dyes about 5% of the stomata and a similar proportion of trichomes showed dye entry in the manner described. In addition many stomata were

Plate 4.12 Location of fluorescent dyes in wild oat leaves:

a. Rhodamine B. (X200)

b. Acridine orange (X200)

c. Coriophosphine (X100).

selectively fluorescent in the guard and accessory cells, although here again there was no evidence of localisation in organelles. Locations of these two dyes are shown in Plates 4.12b (acridine orange) and 4.12c (coriophosphine).

The final two dyes studied, thioflavine T and primuline although from the same group structurally, showed different patterns of location. Thioflavine T, which is cationic showed entry into a few patches of epidermal cells around stomata and trichomes in a similar manner to the acridine dyes (Plate 4.13a). Also the dye was again apparently limited to the cell walls or generally distributed. Primuline caused a very light general fluorescence over the entire area covered by the original drop, possibly due to adsorption onto the surface waxes. In addition some stomata were penetrated (about 5% of total stomata) as shown by increased fluorescence intensity at these sites (Plate 4.13b). The cells in these regions showed no sign of localisation of the dye at sub-cellular organelles, so in this respect primuline was similar to thioflavine T and the acridines.

Overall there seemed to be two major types of entry pattern, one type typified by the fluorones (such as fluorescein) which enter the cells through the plasmalemma and become associated with organelles, the other type typified by the acridines (such as acridine orange) which may be restricted to the cell walls and inter-cellular spaces, or possibly dispersed throughout the cell volume. If dyes showing the second type of pattern do enter the cytoplasm it still appears from the observations that the intensity of fluorescence and hence dye concentration is much greater in the cell walls.

With all dyes studied the stomata were particularly important sites of entry, whether by passage through the pore or by direct entry

Plate 4.13 Location of fluorescent dyes in wild oat leaves:

a. Thioflavine T (X200)

b. Primuline (X200).

into the guard and accessory cells or their walls. The importance of this observation will be discussed in Section 4.5.6.

4.5.4 The entry of fluorescein into various dicotyledonous species

A range of dicotyledonous species were used to study the entry of fluorescein for comparison with the results obtained with wild oat and Zebrina pendula. As with these species 2 ul drops of a solution containing 1.0 $g.l^{-1}$ fluorescein and 0.1% Agral were placed on the leaves of the test plants, and entry of fluorescein was observed after 15-20 minutes, following a wash-off to remove surface deposit.

With the range of species used it was found that the stomatal guard cells were preferential sites of entry of fluorescein as had been observed with wild oats. As before the guard cells showed dye accumulation in nuclei and chloroplasts indicating entry into the cytoplasm. This pattern of entry was observed with radish (Raphanus sativus c.v. Long black Spanish), Stellaria media, Pea (Pisum sativum), Polygonum aviculare, Chrysanthemum segetum, Veronica persica, Viola arvensis and Senecio vulgaris. Photographs of radish, S. media, and pea are shown in Plate 4.14. There was evidence of entry of dye solution into the stomatal cavity with most of the species examined, but as with wild oat this phenomenon only occurred with a small number of stomata and never more than about 5% in total. Examples of stomatal pore entry with radish, P. aviculare, C. segetum and Phaseolus vulgaris are shown in Plate 4.15. Since Phaseolus vulgaris leaves have few stomata on the upper (adaxial) surface the observations on stomatal entry with this species were made on the lower (abaxial) surface as with Zebrina pendula in Section 4.5.2. With all other species observations were made on the upper surface.

Plate 4.14 Location of fluorescein in the leaves of dicotyledonous species:

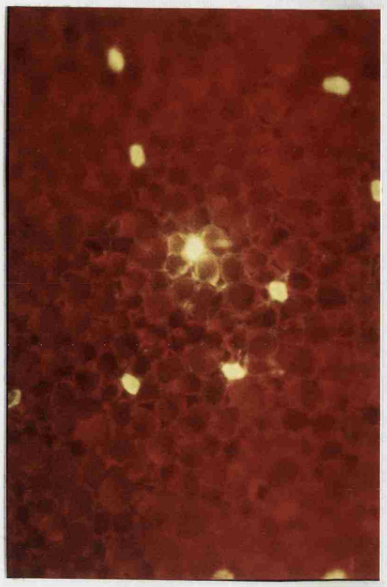
a. Radish (X120)

b. Pea (X120)

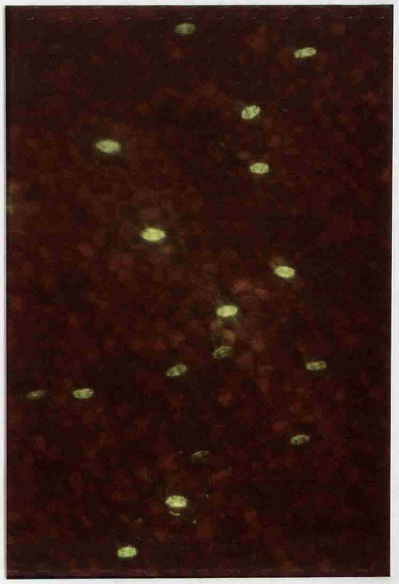
c. Stellaria media (X60)

d. Stellaria media (X180).

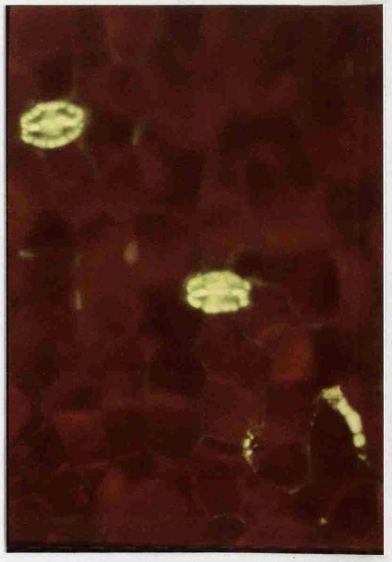
a



b



c



d

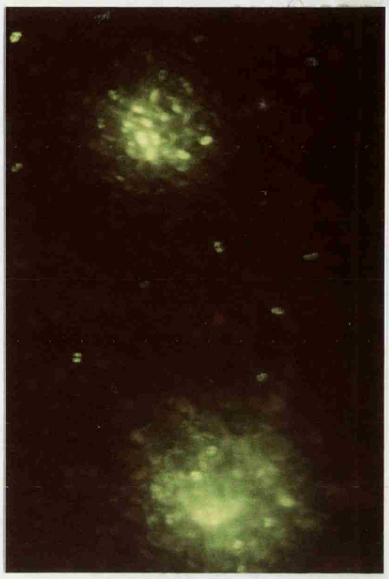
Plate 4.15 Evidence of entry of fluorescein solution through the stomatal pores of four dicotydedonous species:

a. Radish (X60)

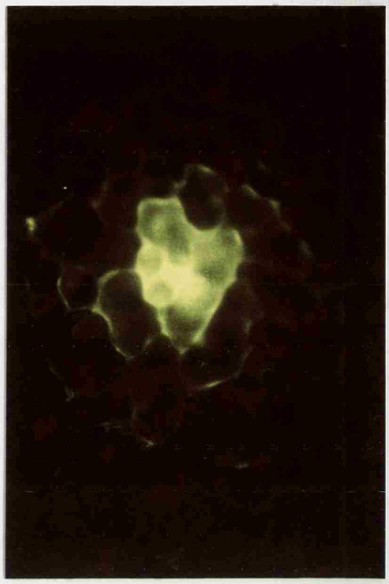
b. Polygonum aviculare (X120)

c. Chrysanthemum segetum (X180)

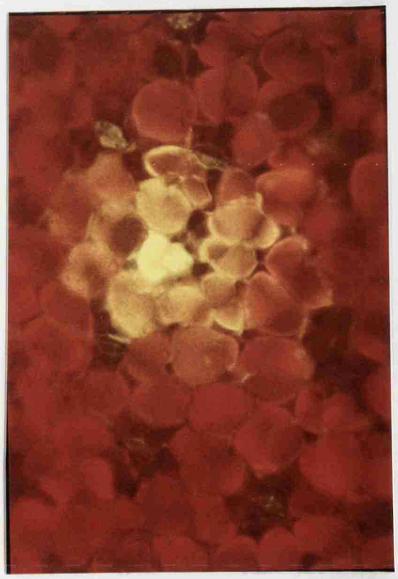
d. Phaseolus vulgaris (X60).



a



b



c

d

As with wild oat trichomes were found to be sites of high uptake of fluorescein. This was demonstrated on the leaves of P. vulgaris where numerous glandular trichomes which line the minor veins were found to take up fluorescein (Plate 4.16a) as was previously recorded by Butterfass (1956). As Butterfass observed, however, the dye did not appear to move from the trichomes into the remainder of the leaf to any obvious degree, due probably to the presence of a ring of cutinization at the base of the trichome. Trichomes are exposed and therefore easily damaged, and it was noticed that damaged trichomes frequently took up more fluorescein. Plate 4.16b shows an example with radish where a considerable mass entry of dye solution occurred at the damaged base of a trichome. Finally, it was observed with most of the species studied that the epidermal cells overlying veins were permeable to fluorescein, as shown with P. vulgaris in Plate 4.16c.

4.5.5 Interpretation of studies of fluorescent dye penetration

A number of factors must be considered when interpreting studies on fluorescent dye penetration.

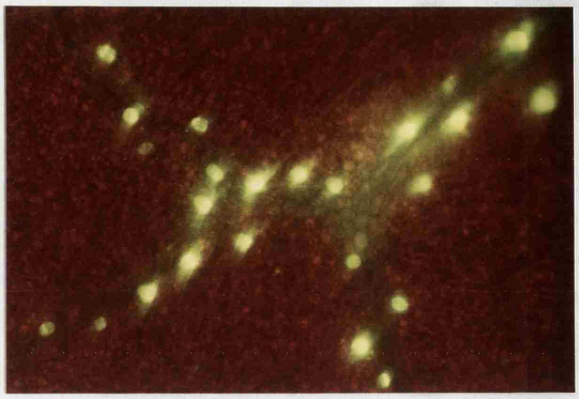
Firstly, all fluorescent dyes are to some extent prone to fading. The result is that as fluorescence is observed the intensity of the emitted light decreases, this being caused by subjecting the dye molecules or ions to light of the excitation wavelength. However, fading does not occur in the absence of excitation light, so in practice it is possible to reduce fading to an acceptable level by keeping the fluorescent material in darkness or subdued light. During observation or photography the time of exposure of the material to the excitation light must be kept to a minimum and standardised. In the present studies, which were not of a quantitative nature, fading caused no major problem.

Plate 4.16 Examples of sites of increased entry of fluorescein in leaves of dicotyledonous species:

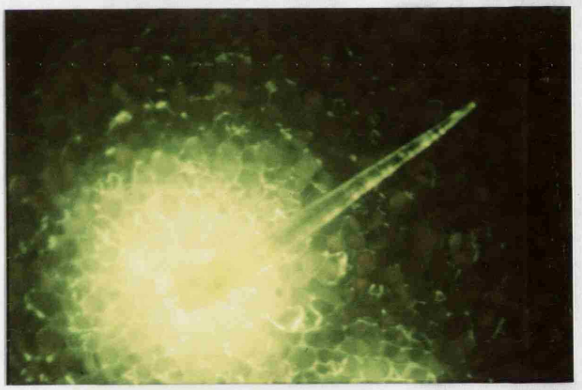
a. trichomes on the underside of a Phaseolus vulgaris leaf. (X60)

b. a radish leaf trichome which had been damaged at its base. (X60)

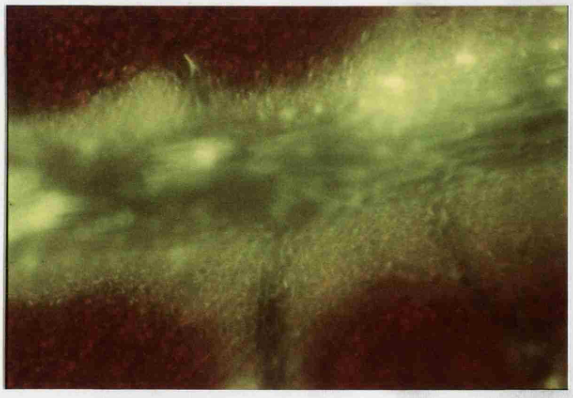
c. a vein on the underside of a Phaseolus vulgaris leaf. (X60)



a



b



c

Secondly, the observation of the site of fluorescent dyes in plant tissues as studied here relied on the fluoresced light travelling through layers of plant material with variable optical properties. Thus the results must to some extent be determined by the processes of reflection, refraction and absorption of the fluoresced light. At the magnifications used in these studies such phenomena were not felt to cause major problems. Occasionally some apparent sources around very brightly fluorescing areas were probably due to reflection by such features as anticlinal cell walls, but in general such effects were fairly obvious and by making a large number of observations it is considered that accurate conclusions were drawn as to the site of fluorescent sources at the cellular level.

A further property of fluorescent dyes which can cause problems, particularly in quantitative work, is that the degree of fluorescence is often subject to quenching. This is the reduction in fluorescent intensity by chemical effects such as changes in pH or adsorption at functional groups on macromolecules or surfaces. This factor is very difficult to eliminate and makes quantitative study of fluorescent intensity very difficult in the presence of biological material. It is encouraging, however, that in the physiological range of pH normally encountered the fluorescent dyes used in this study are not greatly affected by quenching. It is felt that such levels of quenching that might occur are unlikely to have affected the qualitative observations of dye location since the reduction in fluorescence would not be great enough to render the intensity too low for visibility.

Two further points relate to the interpretation of the observations, assuming these to be an accurate assessment of dye location. The first of these points is that the observed location of dye at an

instant in time may not represent the site of entry. To determine this, observations must be made over a period of time so that the first appearance of dye may be determined. Later sites of dye location may be the result of accumulation following movement from a different site of entry which may not itself fluoresce due to rapid movement of dye away from that site.

Finally, the observations of dye entry and location here are intended to give some idea of the sites of entry of foliage-applied herbicides. It is likely that initial entry of dye by mass flow of solution into pores in the plant surface follows very closely that of a herbicide in a solution with similar physical properties. This has been demonstrated by Dybing and Currier (1959, 1961) comparing the distribution of the dye PTS with ^{14}C-labelled maleic hydrazide, distribution of the latter being determined by autoradiography. What is less certain is that the location of dye and herbicide will be the same following penetration through structural layers of the plant surface involving processes other than mass flow. These layers include the cuticular layers, cell walls and membranes such as the plasmalemma. In the preceeding pages it has been shown that even dyes vary considerably in their manner of entry through these layers, so that herbicides will unquestionably vary also. The dyes and herbicides used in the present work are all organic, water soluble materials, but in other respects they vary considerably in their chemistry. Thus it is only possible to observe general principles of the entry of substances into plant surfaces and to attempt to relate differences to chemical structure.

4.5.6 Conclusions

Despite the limitations outlined in the preceding section a

number of significant conclusions can be drawn from these observations on the entry of fluorescent dyes into plant surfaces, with possible implications on the entry of herbicides.

Entry of spray solution in to leaves by mass flow is likely to occur to some extent, and herbicide entering in this way would be more readily absorbed by cells than that remaining on the leaf surface. Likely sites for such en masse entry are open or damaged stomata by pore entry, trichomes, and damaged areas of the epidermis, particularly at leaf margins. It is doubtful whether such entry is of major importance to the overall effect of herbicides since only a small amount of the total applied solution actually enters in this way. In addition it is likely that en masse entry would only occur with some of the drops in a given spray deposit. This would result in locally high concentrations of herbicide in a readily absorbable form within the leaf and may provide an explanation for the observed variability in degree of necrosis by drops on a given area of leaf.

Besides these observations on sites of en masse entry of dye solutions there is some evidence on sites of entry by routes through the cuticular layers and cell membranes. The stomata in particular appear to be important sites of entry of fluorescent dyes of various chemical types into the leaves of a range of plant species. It is evident that this is not due to entry of the solution en masse since the apoplastic dye PTS does not penetrate such sites. The dyes studied varied in their distribution through these stomatal guard and accessory cells. Those dyes which were not localised in sub-cellular organelles may be generally distributed throughout the cells since it has been shown that some pesticides that are only transported in the apoplast were able to thoroughly penetrate the entire cell volume of

potato tuber tissue (Peterson and Edgington, 1976). It was suggested that transport in the symplast did not occur because such pesticides were not concentrated there. A similar explanation may apply to dyes showing no localisation within cells.

It has previously been suggested that stomatal cells are preferential sites of entry of herbicides (Greene and Bukovac, 1977; Sargent and Blackman, 1970). The observations in this study strongly support this view. One possible consequence of this is the injury of death of the stomatal guard and accessory cells soon after the herbicide has been applied, resulting in excessive water loss through the damaged stoma, and consequently the dehydration of adjacent cells. This sequence of events may be responsible for the initiation of necrotic lesions.

5. GENERAL DISCUSSION

The aim of the present study was to investigate the factors which might influence the efficacy of four foliage-applied herbicides at very low volume rates. It is convenient to discuss these factors in sequence.

5.1 Herbicide concentration

When the volume rate is reduced the herbicide concentration is increased, and fewer drops are produced. If the spray drops contain water or other volatile components they will dry on the plant surface leaving a deposit of active ingredient either in an undissolved form or in solution in the involatile components of the spray liquid. Thus at reduced volume rates the drops are more widely spaced and contact a smaller total area of plant surface, and the concentration of the herbicide within the individual drop deposits is increased.

The results of this study suggest that it is the relationship between herbicide concentration and biological efficacy that is most important in determining the suitability of a herbicide for very low volume application. Thus glyphosate, which has been shown to be well suited to very low volume application (Turner and Loader, 1978), was more effective when applied to radish as a smaller number of drops of a more concentrated solution. In contrast difenzoquat performance may be slightly reduced at very low volume rates (Wilson, 1976) and this herbicide was found to be less effective when applied in a more concentrated form (Section 4.3.2.2). With MCPA and paraquat, herbicide concentration in the ranges studied did not significantly influence performance, and both of these herbicides have been applied at very low volume rates without loss in efficacy (Merritt and Taylor, 1977 and

unpublished work). However, many herbicides, including MCPA, are commonly used in mixtures with other herbicides and in such cases the performance of one herbicide at high concentration may influence that of the other components of the mixture.

With difenzoquat it was shown that the reduced performance of higher concentrations was due to the high concentration of herbicides within individual drop deposits, since increased dispersion of a given number of drops did not improve performance. It was found that a major effect of high concentration with this herbicide was necrosis at the site of some or all of the drops within a few days of application. This is assumed to be due to local over-dosing at these sites, since necrosis is not a symptom commonly observed with conventional spraying of this herbicide, although a mottled chlorosis frequently occurs. Whilst this necrosis may simply be the result of general disruption of cells and cuticular layers beneath the drop deposit, it is suggested that this phenomenon is possibly associated with the stomata. Evidence for this is taken from the studies on the entry of fluorescent dyes into plant tissues (Section 4.5) which suggest that stomatal cells may be particularly susceptible to the entry of exogenously applied chemicals. The consequent death or injury of the stomatal cells and adjacent epidermal cells could initiate the formation of necrotic lesions.

It was shown that treatments which caused this local necrosis also reduced the entry into the leaf, and translocation to other plant parts, of ^{14}C-labelled difenzoquat some 3 days after application. There are many possible explanations for this effect of reduced entry and movement of difenzoquat at higher concentrations. Rate of evaporation of the water content of the drops is probably not important, since this occurred within about three minutes of application, during which time

the entry of difenzoquat is negligible (Section 4.2). Also it appears that a difference in the relative proportions of surfactant and herbicide in each drop are not responsible since raising the surfactant concentration pro rata with herbicide concentration was of no benefit to herbicide performance (Section 4.3.2.5). It is possible that entry and hence movement is greater at lower concentrations simply due to the greater area of surface covered by the greater number of drops. However, it is evident that the observed differences in local necrosis between high and low concentrations are involved in the differential uptake, movement and plant response. This may indicate that herbicide resting on the surface of the necrotic lesion is simply unable to penetrate, or move beyond the site of penetration. However, entry and movement after a longer time period (10 days) was equally great with a high and a low concentration of difenzoquat, whilst differences in entry and movement occurred before the full development of necrosis; these results would suggest that uptake and movement are impeded at a stage before the appearance of necrotic symptoms and complete cell desiccation and that such early effects are more important in determining the ultimate biological response with this herbicide. This short term effect on entry and movement could be due to an effect of difenzoquat in reducing the permeability of one or more structural layers, such as the plasmalemma, such that the rate of entry is correspondingly reduced. Alternatively it may be due to a response of the plant cells themselves; an interesting parallel to this phenomenon lies in the localisation of tobacco mosaic virus (TMV) by necrotic lesion formation in hypersensitive hosts. In this case it has been shown that the infected site becomes surrounded by a ring of callose, a polysaccharide produced as a wound response in plants (Schuster and Flemming, 1976; Stobbs and Manocha, 1977). Schuster and Flemming (loc. sit.) showed that this

response also accompanied necrotic lesion formation following treatment of tocacco plants with drops of hydrochloric acid, or touching the leaves with a heated glass rod, and that the resulting lesion was a region of ecessive transpiration which, in their experiments, led to the accumulation at the lesion of labelled calcium reaching the leaves via the petiole.

It seems reasonable that a similar mechanism might apply in the case of difenzoquat injury at locally high doses. Thus initial entry of difenzoquat may cause wound response processes to begin, with the formation of callose restricting the dispersion of difenzoquat away from the site of entry. An increase in the transpiration stream towards the developing lesion could then further reduce dispersion.

These results with difenzoquat show that herbicides may exhibit variation in the relative importance of local and translocated effects with changes in concentration, so that the terms 'contact' and 'translocated' become of questionable usefulness to classify herbicides. The results emphasize in particular the potential danger of misinterpretation of experiments which study a range of volume rates and include reduced herbicide doses; by reducing concentration to achieve the lower doses it is possible to alter the response of the plant to the herbicide, particularly at the site of deposition of individual drops.

Finally, when comparing the results of the retention experiments with those on difenzoquat concentration it is seen that the reduced effect of higher concentrations at very low volume rates may be partially compensated for by an increase in retention of herbidice with the lower volume rates. This may explain the small differences often seen in field experiments comparing conventional and very low volume spraying of difenzoquat. Another factor which would affect results of field

experiments is that of the environmental conditions. It was suggested by Wilson (1976) that variation in the efficacy of very low volume sprays of difenzoquat may have been due to weather conditions, since poorer results were obtained in a dry season. This adds further support to the suggested role of necrosis in difenzoquat performance, since necrosis would tend to be more severe under dry conditions favouring transpiration and reducing plant water content.

5.2 Drop size

The effects of drop size and concentration are likely to be closely linked since both involve variation in the degree of herbicide localisation. This is evident from the results of Section 4.2.1 which showed that drop diameter did not influence the spread factor. Thus if drop diameter were increased by a factor of n, the deposit area would be increased by a factor of n^2; since the volume (and hence the amount of a.i. per drop) would increase by a factor of n^3, the density of a.i. per unit area of drop deposit would increase by a factor of $n^3/n^2 = n$. Therefore if drop diameter is doubled, the density of a.i. per unit area is also doubled.

In this study drop size in the range 200-400 μm was found to affect the performance of difenzoquat, the smaller drops being the more effective. Since this was associated with a reduction in the degree of necrosis at the site of individual drops it was concluded that the mechanism responsible for the difference in performance was similar to that of the effect of concentration with this herbicide.

The other herbicides in this study showed no difference in effect due to drop size in the range studied. It is possible that a further reduction in drop size, perhaps to 100 μm, might bring about a difference in performance with these herbicides, but there is no obvious reason to anticipate such an effect.

Thus from the present results it would appear that a reduction in drop size will only improve biological performance of herbicides such as difenzoquat in which the possibility exists of a local overdosing effect at the concentration involved. This conclusion should be emphasised since drop size is generally considered only in terms of its effect on drop numbers.

Drop size may influence herbicide performance by means other than direct effects on biological activity. A number of authors have described improved retention of small drops (See Section 2.3.2), although Merritt and Taylor (1978) have suggested that this may not apply to the low velocity drops of up to about 300 μm diameter used in CDA techniques, providing that a sufficiently high concentration of a suitable surfactant is used. The use of smaller drops may also increase the likelihood of herbicide reaching any relatively susceptible sites which may present a small target area to the spray, and this aspect will be discussed in the following section.

5.3 Position of deposit

Two aspects of the position of herbicide deposit on the plant surface were considered. The position of spray retained from a controlled drop application was compared with that from a conventional spray, as described in Section 4.1; secondly the variation in plant response to herbicides applied to different positions was studied (described in Section 4.3).

The overall conclusion on the relationship between herbicide performance and position of deposit was that the herbicides used in this study varied in their response to this factor. Thus each herbicide may have a unique set of requirements in relation to its application, which depends on the herbicide mode of action and interactions with the plant.

With wild oats, difenzoquat and paraquat were more effective when applied to the younger leaves. It was found that difenzoquat uptake by leaf 1 was greater than that by leaf 3 after 24 hours, but less after 72 hours. Movement of difenzoquat out of the treated leaf was less from leaf 1 than leaf 3 after 72 hours, and the degree of chlorotic and necrotic symptoms of paraquat which appeared in the young untreated leaves suggested a similar effect with this herbicide. It appears that the various leaves of wild oat can accept a certain maximum dose of these herbicides for optimum biological effect, above which performance is reduced, and that this maximum dose is lower for older leaves. With glyphosate there was no significant effect of leaf age on performance, so that the results with paraquat and difenzoquat are probably due to interaction of the herbicide and plant at the treated site rather than directly to differences in the capacity of the various leaves to transport substances. Retention by the young, erect leaves of wild oat was found to be greater with a conventional spray than with CDA

so this is likely to be a factor contributing to the differences in response with these two application methods.

Increased effect of difenzoquat and paraquat when applied towards the base of the leaf lamina is in agreement with previous findings (Coupland, Taylor and Caseley, 1978) and may be due to proximity to the shoot apex, to differences in surface structure along the leaf lamina or to differences in herbicide effect at the site of treatment, possibly as a consequence of increased dispersion of herbicide within the leaf due to the increased flow of the transpiration stream towards the leaf base.

With radish the foliar leaves were the most important site of spray retention with both conventional and controlled drop applications, followed by the cotyledons, with only small amounts of spray liquid retained on the terminal bud and stem. Paraquat was more effective when applied to the cotyledons, probably because this herbicide moves mostly in the acropetal direction. By contrast MCPA and glyphosate were more effective when applied to the foliar leaves. However there was a difference between these two herbicides in that MCPA was more effective when applied to the mid vein whilst glyphosate was more effective on the lamina between veins, although there is no adequate explanation for this difference.

To summarize, the total amount of spray retained does not appear to be a factor limiting the efficacy of herbicide applications at very low volume rates, although the site of the retained spray on the plant may be affected by changes in application method. In most cases varying the position of the herbicide deposit on the plant resulted in small, though significant differences in biological effect; where differences occur they may be of little consequence when the relative

importance of the sites as targets for spray retention is considered. An example where positional effects may be of importance is the improved retention of spray by the young erect leaves of wild oat from conventional applications compared with CDA, since these leaves are more effective as sites for the application of paraquat and difenzoquat. It is possible that the use of smaller drops with CDA could improve the retention of herbicide by these younger leaves. This result also suggests that it may not be important to achieve movement of spray drops to the older, lower leaves which are often partially hidden by the crop leaves.

5.4 Surfactant concentration

Experiments described in Section 4.3 demonstrated the effects of the concentration of 'Agral' surfactant on the biological performance of MCPA, difenzoquat and paraquat; by applying individual drops effects due to retention were avoided. Under these conditions 'Agral' concentration did not affect the performance of MCPA or paraquat on radish, with concentrations of 'Agral' varying by factors of 1,000 and 100 respectively. With both herbicides the lowest 'Agral' concentration used was 0.01% v/v, this being above the critical micelle concentration so that surface tension remained constant over the range studied. It is possible that herbicide performance might be influenced at lower 'Agral' concentrations but these are unlikely to be used under any normal practical conditions due to the likely reduction in retention.

A reduction in the concentration of 'Agral' to 0.05% v/v, however, caused a very large reduction in the performance of difenzoquat on wild oat; this concentration is one tenth of that recommended by the manufacturers of difenzoquat. In the absence of retention differences this is most likely to be an indication of the role of surfactant in penetration of herbicide into the leaf, either directly through effects of the surfactant on the surfaces layers or through the maintenance of a solution of the herbicide in the surfactant remaining after evaporation of the water from the original drops.

With high concentrations of difenzoquat, particularly those sufficient to cause necrosis, an increase in 'Agral' concentration above that recommended also caused a reduction in the performance of difenzoquat, this being accompanied by an increase in the severity of necrosis. This increase in necrosis was shown to be partly due directly to the high concentration of surfactant, but was largely a result of an increase in

the necrosis caused by the herbicide itself. It is therefore concluded that the higher surfactant concentration caused an increase in the penetration of the herbicide, since severity of necrosis and herbicide entry were found to be correlated in experiments on the effects of herbicide concentration and leaf age (Section 4.4).

It is not evident from the experiments reported in this study whether the fact that 'Agral' concentration influenced difenzoquat performance but not that of MCPA or paraquat was due to the modes of action of the herbicides or differences in surface features between radish and wild oat.

5.5 Concluding Remarks

The experiments of the present study on the effects of drop size, concentration of active ingredient and additives, and positional effects in isolation from each other and from retention effects are the first reported examples which employ drop sizes used in commercial spraying. The development of the single drop applicator was essential for this work, and some device which produces drops within this size range should be recommended for any similar future research programme involving application variables. Some important conclusions have emerged from the results.

The effect of a herbicide at high concentration is the most important factor determining its suitability for very low volume application, this depending largely on whether damage occurs at the site of treatment within a few days after application. A knowledge of the mechanism of such local effects could be used to improve the performance of very low volume sprays and widen the range of herbicides applicable at such rates. Indeed, since the effects at the site of treatment which limit activity appear to take place before the onset of visible necrotic symptoms, it is likely that information gained using high concentrations could also benefit sprays at more conventional volume rates where similar effects may occur, but at sub-necrotic levels. It is most likely that the means to overcome locally damaging physiological effects will be found by research on modification of existing formulations, both with respect to the herbicide molecule itself and to the additive content of the spray. The reduction of volume rate to around 20 $l.ha^{-1}$ or less provides the opportunity to examine formulation variations hitherto regarded as impractical, particularly those involving non-aqueous carrier liquids and expensive additives.

Variation in drop size within the range 200-400 μm does not appear to affect herbicide performance greatly. The drop spreading studies show that to counteract the problems due to increasing density of herbicide deposits as the volume rate is decreased, the drop diameter would need to be halved for each doubling in herbicide concentration. Clearly there is a limit to the extent to which drop size can be reduced whilst maintaining adequate deposition of drops within the target area and retention on plant surfaces. However, future developments could improve retention and drift control with small drops and thus lower the practical limits of drop size. Such developments as the introduction of air streams into sprays (Jegatheeswaran, 1978) and the electrostatic charging of drops (Byass, Lockwood and Andrews, 1979) may help in this way, whilst also altering the pattern of distribution of retained spray on foliage, possibly in a beneficial way.

This study has identified certain potential problems of experimental technique when working with very low volume rates. The use of reduced concentrations of herbicide in order to study reduced doses should be avoided if conclusions are to be drawn relating to the full recommended dose, this being particularly important where a wide range of volume rates are involved; as this study has revealed, the type of plant response may vary with concentration. Secondly the use of large drops, as produced by syringes, may be inappropriate for studies on the entry and movement of foliage-applied herbicides since the results may not relate to those of normal spray drop sizes. It may be particularly important to avoid techniques which significantly alter the rate of drop drying; it appears that under normal environmental conditions spray drops dry quickly leaving a deposit of the active ingredient plus additives, and that most herbicide entry must

therefore take place from such a deposit rather than from an aqueous drop.

It is evident that retention studies are of more value if consideration is given to the distribution of the retained spray, and that this should be linked to a knowledge of the variation in susceptibility of various regions of plant surfaces to the entry and transport of herbicides. Since it appears that all herbicides may have a characteristic set of application requirements peculiar to their modes of action, this work should be extended to cover a wider range of herbicides.

A number of factors which were outside the scope of the present study are nevertheless likely to be important to overall herbicide application requirements. The effects of climatic conditions were largely ignored in this study, but may well influence application requirements. For example the onset of necrosis is likely to be influenced by such factors as humidity, temperature and light intensity. Secondly,the presence of a crop canopy and air movement in the field are likely to influence the retention and drying rates of spray drops. However, crop canopies and air movement in the field are difficult to quantify, and techniques need to be developed which can determine small quantities of spray on the contaminated surfaces of field-grown plants. Finally, the effects of varying the herbicide application method on the tolerance of various crop species and cultivars to herbicides require detailed study; this area also needs special techniques capable of quantifying the effects of sub-lethal doses of herbicide on apical development and relating them to the ultimate crop performance at harvest. Because such techniques remain to be developed crop selectivity was a factor largely ignored in the present study.

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